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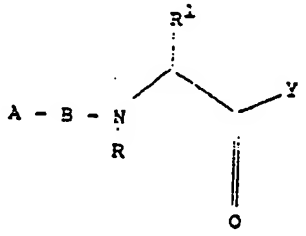

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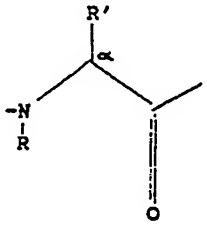

(III)

(57) Abstract

Kininogenase inhibitors, optimally not exceeding the size of a hexapeptide, represented by (II), wherein A and B = amino acyl (including amino acyl analogue) the same or different forming a dipeptide group the amino acid of A carrying a terminal group and being any amino or imino-acid residue (but preferably of D-configuration) and of B being a lipophilic amino-acid residue of D- or L-configuration but not proline or a proline analogue, or a conformational analogue of said dipeptide group wherein the peptide link is replaced by $-CH_2-NH-$ ('reduced'), $-CH(OH)-CH_2-$ ('hydroxy'), $-CO-CH_2$ ('keto'), $-CH_2-CH_2-$ ('hydrocarbon') or other conformational mimic of the peptide bond and in (III) the side chain R^1 is that of a basic amino acid or amino acid analogue (preferably of L-configuration), and R is H or lower alkyl(C_1-C_4) or C^α or the peptide link comprising -N(R)- is replaced leading to a conformational mimic as above; Y = a binding enhancing or carbonyl activating group preferably selected from H (when A or B must be cyclohexylalanine, preferably D if at A or L if at B) or alkyl (C_1-C_{20}) or fluoroalkyl (C_2-C_{12}); substituted oxymethylene; thiomethylene; sulphoxymethylene; sulphonylmethylene; aminomethylene; hydrazino-methylene; $-CH_2$-Het (where Het = a substituted or unsubstituted heterocycle); substituted amino (but when the resulting compound is a secondary alkylamide B must not be phenylalanine); an amino-acid group or its ester or amide; a carboxylic secondary amide or primary amide, when B must be cyclohexylalanine or adamantylalanine or other bulky lipophilic, non-aromatic amino-acid (not Ala Leu Ile Val Nva Met Nle Phe Tyr Trp Nal (1)); tertiary-carboxamide; carboxy-alkyl group or its ester or amide or amino acyl derivative.

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KININOGENASE INHIBITORS

FIELD OF INVENTION

The invention relates to enzyme inhibition and to treatment of disease.

BACKGROUND - KININS

Kinins are natural vasoactive peptides liberated in the body from high molecular weight precursors (kininogens) by the action of selective proteases known as kininogenases.

There is evidence for the involvement of kinins in the following pathological states:

(a) Conditions associated with vasodilatation and hypotension, e.g. septic, anaphylactic and hypovolaemic shock; carcinoid syndrome and dumping syndrome

(b) Conditions involving inflammation, e.g. acute arthritis, pancreatitis, local thermal injury, crush injury and brain oedema

(c) Conditions involving bronchoconstriction, especially for example the initial, acute allergic reaction in asthma

(d) Allergic inflammation, particularly allergic rhinitis and conjunctivitis, together generally known as hay fever, and the bronchial inflammation and consequent occlusion found in the non-acute but serious and even fatal inflammatory phase of asthma.

The kinins (bradykinin, kallidin and Met-Lys-bradykinin) are potent mediators of inflammation. Their main actions are as follows:

(a) They increase capillary permeability which leads to exudate formation and oedema

(b) They are potent vasodilators in arterioles and therefore reduce blood pressure and increase blood flow

(c) They induce pain

(d) They contract bronchial smooth muscle

(e) They activate phospholipase A_2 and thus stimulate the biosynthesis of prostaglandins (PG's) which mediate some of their actions.

In regard to prostaglandins, it may be noted that certain actions of kinins, particularly pain and vascular permeability above, are potentiated by PG's, although PG's themselves do not cause pain nor do they induce vascular permeability at the concentrations found in inflamed tissue. PG's therefore act as either mediators or potentiators of kinins.

In spite of the above knowledge of kinins and their actions, relatively little attention has been paid to reduction of their action. In asthma treatment for example clinical attention is primarily directed to the acute bronchoconstrictive reaction, for which there are effective drugs. Deaths continue to occur from the gradually developing bronchial occlusion, and at present not only are there no clinically effective inhibitors of kinin release available but the concept of kinin release inhibition, at least in treating allergic inflammation, appears to be new. The only substance that is in fact a kinin release inhibitor and has attained clinical significance is aprotinin ('Trasylol', Bayer, trade mark), a proteinase inhibitor isolated from bovine tissues (lungs, lymph nodes and pancreas). It is a strongly basic protein (pI = 10.5) of MW = 6,500 comprising a single peptide chain of 58 residues. However, aprotinin is primarily a trypsin inhibitor (Ki = 10^{-13}M) and is some 10^6-times less active against kinin release. It has been found marginally beneficial in acute pancreatitis, a serious condition, where it inhibits the activation by trypsin of zymogens of pancreatic serine proteinases, and in traumatic - haemorrhagic shock. Aprotinin has to be administered parenterally, and it frequently produces a painful reaction at the injection site.

BACKGROUND - KININOGENASES

The kininogenases are serine proteinases, that is to say proteinases in which the hydroxy group of a serine residue is the nucleophile involved in forming the substrate transition state. They liberate the kinins (bradykinin, kallidin) from the kininogens by limited proteolysis. There are several kinds of kininogenase:-

(a) Tissue kallikrein (TK, also called glandular kallikrein GT or urinary kallikrein UK) which is found in the pancreas, salivary glands, intestines, kidney and urine. It has MW = 30,000 and acts preferentially on low molecular weight kininogen (LMWK) to release the kinin kallidin (KD). Tissue kallikrein has no potent and fast acting endogenous inhibitor present in plasma.

(b) Plasma kallikrein (PK) occurs in plasma as an inactive zymogen which is activated by Factor XIIa, and is part of the intrinsic coagulation cascade. It has MW = 100,000 and its preferred substrate is high molecular weight kininogen (HMWK) from which it releases bradykinin (BK). Plasma kallikrein is rapidly and effectively inhibited in plasma, by endogenous inhibitors known as c1-inactivator and α_2-macroglobulin.

(c) Mast cell tryptase which, while not as active as the kallikreins in kinin release, we have found to occur in large amounts in the mast cells of the lung tissue of asthmatics.

BACKGROUND - KININOGENS

The kininogens which are the natural substrates for the kininogenases (they act also as potent inhibitors, Ki approx. 10^{-11}, of cysteine proteinases such as cathepsins B, H and L, calpain and papain) occur in two types:

(a) Low molecular weight kininogen (LMWK) with molecular weight in the range 50,000 - 70,000 depending on species of origin and degree of glycosylation.

(b) High molecular weight kininogen (HMWK) with molecular weight in the range 88,000 - 114,000 which, in addition to serving as an alternative precursor of kinins and a cysteine proteinase inhibitor, also plays an obligatory role with plasma kallikrein in the initiation of the intrinisic coagulation cascade.

The two kininogens, whose mRNA's are transcribed from the same gene, have identical primary sequences throughout the N-terminal or heavy chain (H-chain) region, the kinin region and the first twelve amino acids of the C-terminal or light chain (L-chain). At this point their structures diverge, HMWK having a longer L-chain (MW approximately 45K) than LMWK (4.8K).

The cleavage of human HMWK by plasma kallikrein is for example shown schematically in Fig. 1, with details of the sequence at the cleavage sites in Fig. 2 and a more detailed sequence in Fig. 3 where the conventional numbering of residues adajcent to a cleavage site is shown for cleavage site I. After excision of one or other kinin sequence, the H- and L-Chains are held together by a single disulphide bridge:-

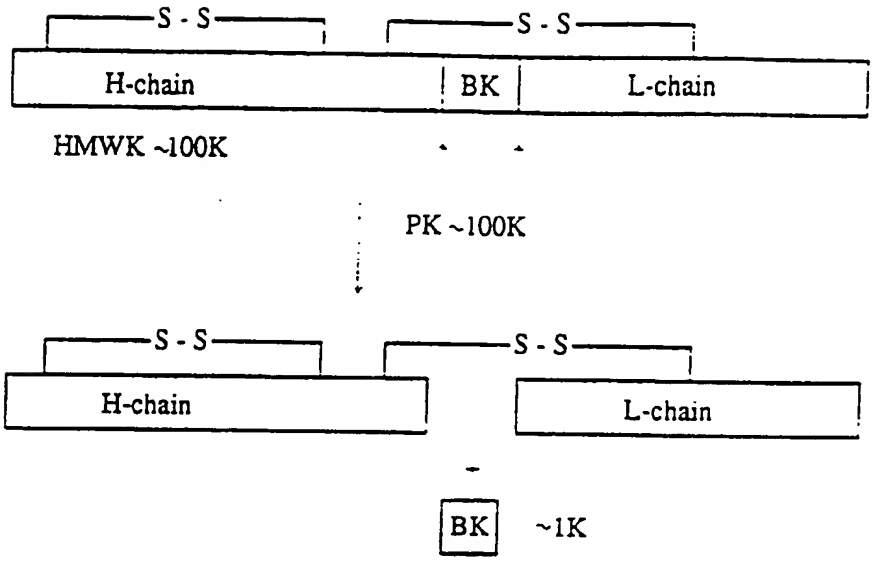


Figure 1. Cleavage of HMWK by PK: Overall scheme

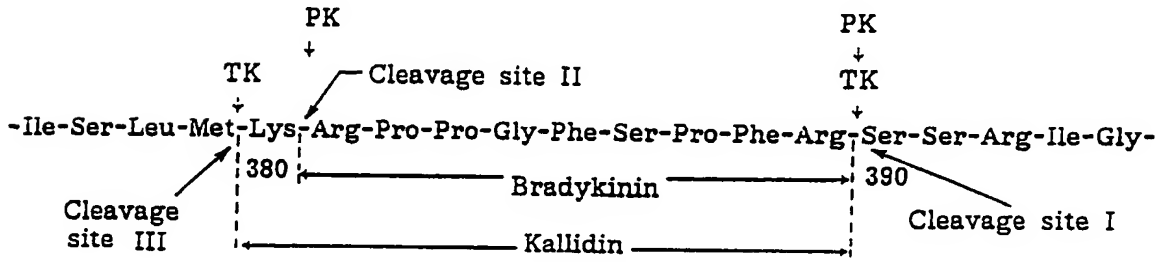


Figure 2. Cleavage of human kininogens by PK and TK: Details of sequence

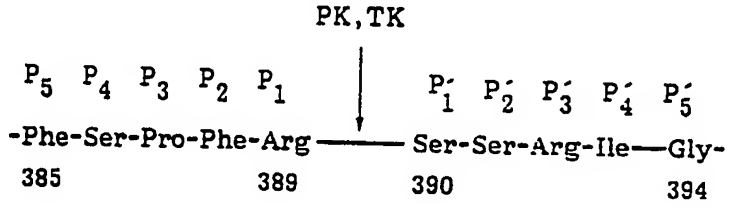


Figure 3. Sequences flanking cleavage site I in human HMWK

As shown, plasma kallikrein and tissue kallikrein act at a single site to free the kinin C-terminal site, cleaving between residues 389 and 390, but at sites one residue apart, either side of residue 380, to free the N-terminal of bradykinin (by PK) or kallidin (by TK).

The role of PK and HMWK as clotting factors in the intrinsic cascade does not involve the enzymatic release of kinins. However many of the effects of PK and all those of TK do involve such release, being mediated by the kinins released from the respective substrates HMWK and LMWK through selective proteolysis.

INDICATIONS

The main clinical indications for kininogenase inhibitors are inflammatory conditions, particularly allergic inflammation (e.g. asthma and hay fever). A fuller list of indications is given below:

(1) Allergic inflammation (e.g. asthma, rhino-conjunctivitis [hay fever], rhinorrhoea, urticaria

(2) Inflammation (e.g. arthritis, pancreatitis, gastritis, inflammatory bowel disease, thermal injury, crush injury, conjunctivitis)

(3) Smooth muscle spasm (e.g. asthma, angina)

(4) Hypotension (e.g. shock due to haemorrhage, septicaemia or anaphylaxis, carcinoid syndrome, dumping syndrome)

(5) Oedema (e.g. burns, brain trauma, angioneurotic oedema whether or not as a result of treatment with inhibitors of angiotensin converting enzyme)

(6) Pain and irritation (e.g. burns, wounds, cuts, rashes, stings, insect bites)

STATEMENT OF INVENTION

In one aspect the invention provides a method of treatment (including prophylactic treatment) of an inflammatory or other condition set out in the indications above, particularly an allergic inflammatory condition, wherein an effective amount of a peptide or peptide-analogue kininogenase inhibitor is administered topically or systemically to a patient suffering from or at risk of the condition. It is believed that for optimum activity, administrability and stability in the body the compounds should not exceed the size of a hexapeptide, that is to say should not comprise more than six amino acid or amino acid analogue residues; the presence of further residues, particularly in a pro-drug from which residues are cleaved in the body to give the compound primarily exerting the desired effect, is however not excluded.

Particularly, the invention provides a method of treatment of the allergic inflammatory phase of asthma, wherein an effective amount of a kininogenase inhibitor such as a mast cell tryptase inhibitor is administered topically or systemically to a patient suffering from or at risk of the condition.

The invention extends further to a method of preparation of a medicament for the topical or systemic treatment (including prophylactic treatment) of conditions as above particularly for allergic inflammatory conditions and especially for asthma as above, wherein a kininogenase inhibitor is associated with a pharmaceutically acceptable diluent or carrier to constitute said medicament.

In the above, the kininogenase inhibitor is conveniently

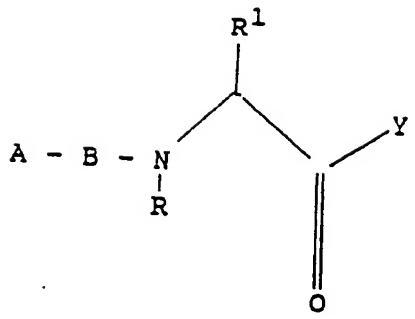
but not essentially of the novel kind now described in which in another aspect, without limitation to any particular clinical indication, the invention provides synthetic, low molecular weight compounds that selectively inhibit kininogenases and thus block the release of kinins from kininogens. The inhibitors are peptide analogues, desirably (as above) not exceeding the size of a hexapeptide in terms of amino acid or analogue residues, based on the known amino acid sequence of the kininogens at cleavage site I, which analogues have sufficient similarity to the cleavage site sequence to bind to the active site of the kininogenase but are not hydrolysable and therefore remain bound, inactivating the enzyme.

The inhibitors are essentially of the structure below, in which A represents the P_3 residue, B the P_2 residue, C the P_1 residue and Y a carbonyl-activating or binding group the structure being:-

A - B - C - Y I

where A, B and C are amino acyl or amino acyl analogue groups linked by peptide bonds or conformational analogues thereof giving a peptide mimic. Other residues in addition to these essential ones may of course be present, including amino acyl or amino acyl analogue residues.

In more definitive terms the compounds are represented by

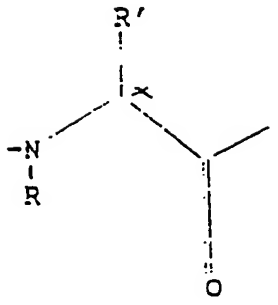


II

wherein

A and B = amino acyl (including amino acyl analogue) the same or different forming a dipeptide group the amino acid of A optionally carrying a terminal group (other than hydrogen) and being any amino or imino-acid residue (but preferably of D-configuration) and of B being a lipophilic amino-acid residue of D- or L-configuration but not proline or a proline analogue, or a conformational analogue of said dipeptide group wherein the peptide link is replaced by $-CH_2-NH-$ ('reduced'), $-CH(OH)-CH_2-$ ('hydroxy'), $-CO-CH_2-$ ('keto'), $-CH_2-CH_2-$ ('hydrocarbon') or other conformational mimic of the peptide link

and in:-



III

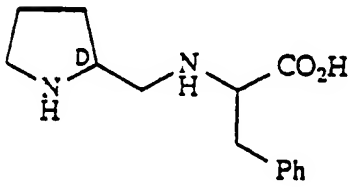
the side chain R^1 is that of a basic amino acid or amino acid analogue (preferably of L-configuration) and R is H or lower alkyl (C_1 - C_4) or C^α or the peptide link comprising -N(R)- is replaced leading to a conformational mimic as above. For example C^α may be replaced by nitrogen.

Y = a binding enhancing or carbonyl activating group for example selected from H (but only if A or B is cyclohexylalanine, preferably D if at A or L if at B) or alkyl (C_1 - C_{20}) or fluoroalkyl (C_2 - C_{12}); substituted oxymethylene; thiomethylene; sulphoxy-methylene; sulphonylmethylene; aminomethylene; hydrazino-methylene; $-CH_2$-Het (where Het = a substituted or unsubstituted heterocycle); substituted amino (but when the resulting compound is a secondary alkylamide B must not be phenyl-alanine); an amino-acid group or its ester or amide; a carboxylic secondary amide or primary amide, when B must be a bulky lipophilic, non-aromatic amino-acid e.g. cyclohexylalanine, adamantylalanine (not Ala Leu Ile Val Nva Met Nle Phe Tyr Trp Nal (1));

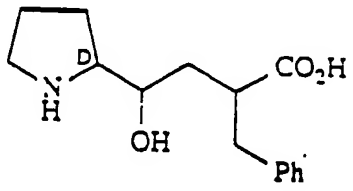
tertiary-carboxamide; carboxy-alkyl group or its ester or amide.

In the above context substituents are suitably common functional groups that increase binding affinity to the enzyme and/or improve pharmacological properties. Further in considering conformational analogues or mimics a dipeptide mimic is a structure containing non-natural amino acid (amino acid analogue) residues or which is non-peptidic and which in I holds the side-chains of A and B or B and C or all of them in a conformation similar to that present in the parent peptide when bound to the active site of the enzyme. It may also contain features favourable for other interactions with the enzyme, e.g. hydrogen bonding. A mimic may be chosen from the published work on such analogues.

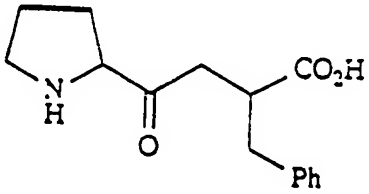
For example, the following are mimics of the dipeptide DPro-Phe (Ph may be replaced by $-CH_2Ph$):-



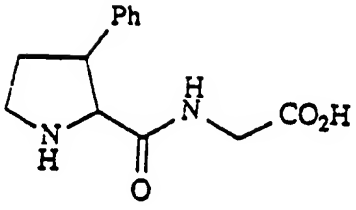
(i) 'Reduced' mimic



(ii) 'Hydroxy' or hydroxyethylene mimic



(iii) 'Keto' or ketomethylene mimic



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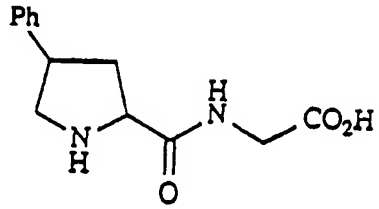
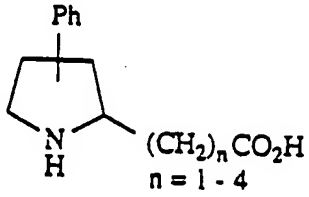


(v)



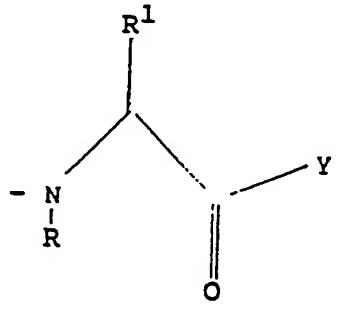
(vi) ('Hydrocarbon' mimic)

Figure 4, DPro-Phe mimics

The preferred compounds represented by the above general formula are now considered.

Preferred residues for A are imino-acids, (e.g. D-proline or an analogue of proline e.g. pipecolinic acid, azetidine carboxylic acid etc.); lipophilic amino acids (e.g. DPhe, DCha, DChg); strongly basic amino acids (e.g. D-Arg or a guanidinophenylalanine) and for B they are L-Phe, L-Cha, L-αNal, L-Tal, L-(4F)Phe L-(NMe)Phe or other substituted phenylalanines. A and B may also be the N-alkyl (C_1-C_4) or Cα-alkyl (C_1-C_7 e.g. methyl, benzyl) analogues of these amino acids. Suitable terminal groups for A include lower alkyl (preferred) or acyl (not excluding amino acyl), alkyl sulphonyl (straight chain or branched or cyclic), amino-alkyl, carboxy alkyl, hydroxy alkyl or any other common protecting group encountered in peptide chemistry.

Groups suitable as group Y are specific to the present invention in that they are part of the structure giving the required binding to the active site and are not merely non-interfering end groups. They form a binding group which increases affinity to the enzyme and/or a group which activates the adjacent carbonyl by rendering it more electrophilic. Specific groups are included in the following formula:



IV

where in a peptide link to residue B the α-nitrogen may be free or substituted for example by methyl or other C_1-C_4 alkyl and thus R^1 and R are as before but particularly R^1 = 3-guanidinopropyl or other guanidinoalkyl group or an amidino-alkyl or aminoalkyl group, also para- or meta substituted guanidino or amidino-benzyl or protected forms of the above (the basic nitrogens may also be alkylated e.g. with Me, Et), and where

Y = groups as given below, first in more general terms and then in terms of more detailed preferences, subject in both cases to the provisos expressed in defining the compounds of the invention earlier. The detailed preferences are given in groups under roman numerals, which are also indicated, in brackets, with the first listing which is:-

(I) Y = H (representing aldehydes) or alkyl including fluoroalkyl (representing ketones)

(II-III-IV) Y = $-CH_2Q$

where Q = $-OR^2$ or $-SR^2$ or $-SOR^2$ or $-SO_2R^2$ or

$-NHR^2$ or $-N(R^4)(R^5)$ or $-\textcircled{D}$

(note that $-\textcircled{D}$ represents a ring in which D is an atom of that ring)
wherein R^2, R^4 and R^5 are as below

(V-VI) Y = $-CH_2\ CHR^6\ CON\begin{matrix}R^4\\R^5\end{matrix}$ or

$-CH_2\ CHR^6\ CO-D$

wherein R^4, R^5 and R^6 are as below

(VII-VIII) Y = amino acyl or a group forming a substituted amide or hydrazide

(IX-X-XI) Y = a group forming an α-keto amide e.g. $-COR^9$ or -CO-D or

$-CON\begin{matrix}R^4\\R^5\end{matrix}$

and in which further:-

R^2 = alkyl or substituted alkyl including aryl or aryl alkyl and $-CH_2R^3$ where R^3 = fluoroalkyl

R^4 and R^5 the same or different but not both hydrogen = H or C_1-C_{20} alkyl (which may be further substituted), acyl or alkyl sulphonyl

-D is a heterocyclic ring (D = nitrogen or carbon in Group IV and N in Groups VI and X) optionally unsaturated and optionally with further hetero atoms and substituents

R^6 = hydrogen, alkyl, hydroxyalkyl, aminoalkyl, alkylaminocarbonyl

R^9 = $-NH_2$ as such or alkylated, or amino acyl

The listing of more detailed preferences, again within the provisos expressed earlier, is:-

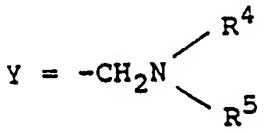
Group I

Y = H; alkyl including branched alkyl (C_1-C_{20}); aryl alkyl; or cycloalkyl (C_1-C_{20}); perfluoroalkyl or partially fluorinated alkyl (C_2-C_{12}); [e.g. Y = Me; $-CH(CH_2CH_2CH_2CH_3)_2$; $-CH(CH_2CH_2CH_2CH_3)CH_2$-cyclohexyl; $-CH_2CF_2CF_2CF_3$; $-CF_2CH_2CH_2CH_3$].

Group II

Y = $-CH_2QR^2$ where Q = O, S, SO, SO_2, NH and where R^2 = Alkyl, branched alkyl or acyl (C_1-C_{12}); or cycloalkyl (C_1-C_{20}); or aryl or aryl alkyl; or $-CH_2-R^3$ where R^3 = perfluoroalkyl or partially fluorinated alkyl, branched or not (C_1-C_{12}).

Group III



R^4, R^5 the same or different = alkyl, branched alkyl, cycloalkyl, acyl, alkylsulphonyl, carboxyalkyl (the carboxyl group may be further derivatized to form an ester or amide with an amino-acid or dipeptide), carbamoyl, sulphamoyl, N-dialkylamino-, arylalkyl, haloalkyl including fluoroalkyl, cyanoalkyl, alkoxyalkyl,

hydroxyalkyl, mercaptoalkyl, aminoalkyl and derivatives thereof e.g. esters, amides and thioesters; or one of R^4 or R^5 = hydrogen

Group IV

$$Y = -CH_2 - D$$

where D = nitrogen or carbon and -D is a saturated or unsaturated heterocyclic ring or a bicyclic ring system, each is 5 - 8 membered, where there may be other hetero-atoms (N, S, 0) and carbons or nitrogens may optionally be substituted by alkyl, branched alkyl, cycloalkyl, carboxyalkyl, carboxy (attached to carbon), amino, alkoxy, alkoxymethyl or (carbon) as carbonyl or other groups beneficial for interaction with the enzyme.

Group V

$$Y = -CH_2CH(R^6)CONR^4R^5$$

R^4, R^5 as defined in Group III.
R^6 = hydrogen lower alkyl, branched alkyl, cycloalkyl, hydroxyalkyl, amino-alkyl, alkylaminocarbonyl.

Group VI

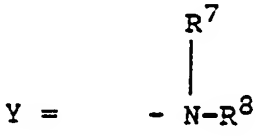
$$Y = -CH_2CH(R^6)COD$$

R^6 as defined in Group V and - D
as defined in Group IV (but with D = N)

Group VII

Y = an amino-acid residue or any amide (secondary or tertiary) or ester of that residue, L or D configuration. Preferred residues are of lipophilic amino-acids e.g. norleucine, cyclohexylalanine, homocyclohexylalanine, cyclohexylglycine, ^{tert}butylglycine.

Group VIII



R^7 = H (when however B is not phenylalanine unless R^8 is carboxylalkyl or derivatized carboxylalkyl); or alkyl, branched alkyl (C_1 - C_{12}), cycloalkyl (C_1-C_{20}) carboxyalkyl or bis(carboxyl)alkyl, which may be derivatized at the carboxyl group to form an amide e.g. with an amino-acid (preferred is arginine) or a substituted amine; N'-dialkylamino; N'-alkylamino-;

R^8 = R^7 the same or different but excluding H.

Group IX

Y = -CO-R^9 but only if B is a bulky non-aromatic lipophilic amino acid or its N^{α} alkyl (C_1 - C_4) derivative (e.g. cyclohexylalanine but excluding Ala, Leu, Ile, Val, Nva, Met, Nle, Phe, Tyr, Trp, Nal(1) and their N-methyl derivatives) where R^9 = NH_2, N'-alkylamino (where the alkyl groups include branched and/or cycloalkyl); an amino-acid residue.

Group X

$$Y = -CO-\textcircled{D}$$

$-\textcircled{D}$ as defined in Group IV (but D = N)

Group XI

$$Y = -CO-NR^4R^5$$

R^4,R^5 as defined in Group III, but not H

The following examples illustrate the invention. They are given in the form of:-

- Nine tables of compounds with reference number, structure, molecular ion as determined by FAB (fast atom bombardment) spectrometry and class of compound (the same as the 'groups' referred to earlier herein)
- Eight detailed examples of synthesis
- Twelve synthesis schemes, as referred to in the detailed examples
- Table of abbreviations
- Description of in vitro tests of inhibition of kininogenases and in vivo tests of efficacy against asthma

All structures of intermediates were verified by NMR.

TABLE 1

Aldehydes

				[M+H]+	Class
5	Me-DPhe	Cha	Arg-H	473	I

TABLE 2

Thiomethylene Analogues and Sulphomethylene Analogues

				[M+H]+	Class
17	H-DPro	Phe	Arg $CH_2S(CH_2)_4Me$	519	II
60	H-DPro	Phe	Arg CH_2S^nBu	505	II
61	H-DPro	Phe	DLArg $CH_2SO_2{}^nBu$	537	II

TABLE 3

Ethers

				[M+H]+	Class
23	H-DPro	Phe	Arg $CH_2OCH_2(CF_2)_3CHF_2$	647	II
62	H-Pro	Phe	Arg $CH_2OCH_2CF_3$	515	II
63	Boc-DPro	Phe	DLArg $CH_2OCH_2CF_3$	615	II
64	H-DPro	Phe	DLArg $CH_2OCH_2CF_3$	515	II
65	H-DPro	Cha	DLArg $CH_2OCH_2CF_3$	521	II
66	H-DPro	Phe	Arg $CH_2OCH(Me)CF_2CF_2CF_3$	629	II
67	H-DPro	Phe	Arg $CH_2OCH_2CF_3$	515	II
68	H-DCha	Phe	Arg $CH_2OCH_2CF_3$	571	II
69	H-DArg	Phe	Arg $CH_2OCH_2CF_3$	574	II
70	H-DChg	Phe	Arg $CH_2OCH_2CF_3$	557	II
54	H-DPro	Phe	Arg $CH_2O(CH_2)_5CH_3$	517	II
55	H-DPro	Phe	Arg CH_2OPh	509	II

TABLE 4

Aminomethylene and related analogues

				[M+H]$^+$	Class
31	H-DPro	Phe	Arg $CH_2N[(CH_2)_5Me]_2$	600	III
71	H-DPro	Phe	DArg CH_2(RS)Tha	556	IV
72	H-DPro	Phe	Arg CH_2(RS)Tha	556	IV
73	H-DPro	Phe	Arg CH_2 1-Pip-3-(RS)CO_2Et	572	IV
74	H-DPro	Phe	DLArg CH_2 1-Pip-3-(RS)$CH_2O(CH_2)_3Me$	586	IV
75	H-DPro	Phe	DLArg CH_2 1-Pip-3-(RS)CH_2NEt_2	585	IV
76	H-DPro	Phe	DLArg CH_2 1-Pip-3-$CONEt_2$	599	IV
77	H-DPro	Phe	DLArg CH_2 1-Pip-3-(RS)$CONH^nBu$	599	IV
78	H-DPro	Phe	DLArg CH_2 1-Pip-3-(RS)$CON(^nBu)_2$	655	IV
79	H-DPro	Phe	DLArg CH_2 1-Pip-4-(1'-Pip)	583	IV
80	H-DPro	Phe	Arg CH_2(NMe)AhaNH^nBu	615	IV
81	H-DPro	Phe	Arg CH_2-Abn	540	IV
82	H-DPro	Phe	Arg CH_2-Hyp(O^nBu)NHEt	629	IV
83	H-DPro	Phe	Arg CH_2-Sar Pro NHEt	628	III
84	H-DPro	Phe	Arg CH_2-^{t}Hyp(O^nBu)$^{\beta}O^nBu$	644	IV
85	H-DPro	Phe	DLArg CH_2Pic NEt_2	641	IV
86	H-DPro	Phe	DLArg $CH_2{}^t$DHyp(O^nBu)$^{\beta}O^nBu$	644	IV
87	H-DPro	Phe	DLArg $CH_2Pro^{\beta}CONEt_2$	599.9	IV
88	H-DPro	Phe	DLArg $CH_2$1-Pip-3(RS)CO_2H	544	IV
89	H-DPro	Phe	Arg $CH_2$1-Pip-3(RS)CH_2CONEt_2	613	IV
90	H-DPro	Phe	Arg $CH_2N(CH_2Ch)(CH_2)_5Me$	612	III
91	H-DPro	Phe	DLArg $CH_2N(Oc)_2$	657	III
92	H-DPro	Phe	Arg CH_2N(Et)Ch	542	III
93	H-DPro	Phe	Arg $CH_2N(Me)(CH_2)_4NH_2$	517	III
94	H-DPro	Phe	Arg $CH_2N(Me)^nBu$	502	III
95	H-DPro	Phe	DLArg $CH_2N^nBu_2$	544	III
96	H-DPro	Phe	DLArg $CH_2N[^nBu]SO_2{}^nBu$	594	III
97	H-DPro	Phe	DLArg $CH_2N[^nBu](CH_2)_3CONH_2$	573	III
98	H-DPro	Phe	DLArg $CH_2N[^nHex](CH_2)_7NH_2$	629.5	III
99	H-DPro	Phe	DLArg $CH_2N[^nHex](CH_2)_5NH_2$	601	III
100	H-DPro	Phe	DLArg $CH_2N[^nHex](CH_2)_3NH_2$	573	III
101	H-DPro	Phe	DLArg $CH_2N[^nBu](CH_2)_4Ph$	620	III

TABLE 4 (cont.)

Aminomethylene Ketones and related analogues

				[M+H]$^+$	Class
102	H-DPro	Phe	DLArg $CH_2N[^nHex](CH_2)_6CONH_2$	643	III
103	H-DPro	Phe	DLArg $CH_2N[^nHex](CH_2)_6NH_2$	615	III
104	H-DPro	Phe	DLArg $CH_2N[^nHex](CH_2)_7OH$	630	III
105	H-DPro	Phe	DLArg $CH_2N[^nHex](CH_2)_7NHAc$	671	III
106	H-DPro	Phe	DLArg $CH_2N[^nHex](CH_2)_6CONHEt$	671	III
107	H-DPro	Phe	DLArg $CH_2N[^nHex](CH_2)_6CO_2Me$	658	III
108	H-DPro	Phe	DLArg $CH_2N[^nHex](CH_2)_6CO_2H$	644	III
109	H-DPro	Phe	DLArg $CH_2N[^nBu](CH_2)_3CONEt_2$	629	III
110	H-DPro	Phe	DLArg $CH_2N[^nBu](CH_2)_3CONHEt$	601	III

TABLE 5

Keto Isostere Containing Analogues

				[M+H]$^+$	Class
40	H-DPro	Phe	DLArg^{K}Gly Pro-NHEt	599	VI
111	H-DPro	Phe	Arg^{K}Gly Pro-NHEt	599	VI
112	H-DPro	Phe	Arg^{K}Gly Arg-NH_2	530	V
113	H-DPro	Phe	DLArg^{K}Gly Ala-NH_2	545	V
114	H-DPro	Phe	DLArg^{K}Gly Aha-NH_2	587	V
115	H-DPro	Phe	DLArg^{K}Gly Aha-NH^nBu	643	V

TABLE 6

Substrate Analogues

				[M+H]$^+$	Class
45	H-DPro	Phe	Arg Chg-NH_2	557	VII
116	CPr-CO	Phe	Arg Chg-NH_2	*	VII
117	$MeSO_2$ DPro	Phe	Arg Chg-NH_2	635	VII
118	MeCO DPro	Phe	Arg Chg-NH_2	599	VII
119	H-DArg	Phe	Arg Ser- NH_2	*	VII
120	H-DCha	Phe	Arg Ser- NH_2	*	VII
121	H-DPhe	Phe	Arg Ser- NH_2	*	VII
122	H-DPic	Phe	Arg Ser- NH_2	*	VII

TABLE 6 (cont.)

Substrate Analogues

				[M+H]$^+$	Class
123	H-DPic	Phe	Arg Chg-NH_2	*	VII
124	H-DPro	Phe	Arg Ser-NH_2	*	VII
125	H-DPro	Cha	Arg Ser-NH_2	*	VII
126	H-DPro	Cha	Arg Gly-NH_2	*	VII
127	H-DPro	Phe	Arg Ser-Arg-NH_2	*	VII
128	H-DPro	Phe	Arg Lys-NH_2	*	VII
129	H-DPro	Phe	Arg Aha-NH_2	*	VII
130	H-DPro	Phe	Arg Phe-NH_2	565	VII
131	H-DPro	Phe	Arg Leu-NH_2	531	VII
132	H-DPro	Phe	Arg Ile-NH_2	531	VII
133	H-DPro	Nal	Arg Ser-NH_2	*	VII
134	H-DPro	Phe	Arg DAha-NH_2	531	VII
135	H-DPro	Phe	Arg Aha-$NH(CH_2)_3Me$	587	VII
136	H-DPro	Phe	Arg Nleucinol	518	VII
137	H-DPro	Phe	Arg SerO^nBu NH_2	561	VII
138	H-DPro	Phe	Arg Cha-NH_2	571	VII
139	H-DPro	Phe	Arg Ada-NH_2	623	VII
140	H-DPro	Phe	Arg Hch-NH_2	585	VII
141	H-DPro	Nal	Arg Cha-NH_2	*	VII
142	H-DPro	Cha	Arg Cha-NH_2	*	VII
143	H-DPro	PhepNO_2	Arg Ser-NH_2	*	VII
144	H-DPro	Nal	Arg Ile-NH_2	*	VII
145	H-DPro	Phe	Arg Ile Pro-NH_2	*	VII
146	H-DPro	Phe	Arg Aha Pro-NH_2	*	VII
147	H-DPro	Nal	Arg Aha-NH_2	*	VII
148	H-DPro	Cha	Arg Npg-NH_2	*	VII
149	H-DPro	Cha	Arg Hch-NH_2	*	VII
150	H-DPro	Nal	Arg Hch-NH_2	*	VII
151	H-DPro	Phe	Arg Npg-NH_2	545	VII
152	H-DPro	Phe	Arg Chg-1-Pip	625	VII
153	H-DPro	Phe	Arg Chg-$NH(CH_2)_5Me$	641	VII
154	H-DPro	4-Fph	Arg Chg-NH_2	575	VII

* Satisfactory amino acid analysis obtained

TABLE 7

Amides

				[M+H]$^+$	Class
47	H-DPro	Phe	Arg N[$(CH_2)_5$Me]$(CH_2)_3$Ch	626	VIII
155	H-DPro	Phe	Arg N(Me)^{n}Bu	488	VIII
156	H-DPro	Phe	Arg $NH(CH_2)_3CO$ Arg-NH_2	*	VIII
157	H-DPro	Phe	Arg $NH(CH_2)_3NH_2$	475	VIII
158	H-DPro	Phe	Arg $NH(CH_2)_4CO$ Arg-NH_2	*	VIII
159	H-DPro	Phe	Arg $NH(CH_2)_4NH_2$	*	VIII
160	H-Pro	Phe	Arg $NH(CH_2)_5CO$ Arg-NH_2	687	VIII
161	H-DPro	Phe	Arg $NH(CH_2)_5NH_2$	503	VIII
162	H-DPro	Phe	Arg $NH(CH_2)_6CO$ Arg-NH_2	701	VIII
163	H-DPro	Phe	Arg $NH(CH_2)_7CO$ Arg-NH_2	715	VIII
164	H-DPro	Phe	Arg $NH(CH_2)_7CONH(CH_2)_3Me$	615	VIII
165	H-DPro	Phe	Arg $NH(CH_2)_7NHAc$	573	VIII
166	H-DPro	Phe	Arg $NH(CH_2)_7NH_2$	531	VIII
167	H-DPro	Phe	Arg $NH(CH_2)_7CONH_2$	559	VIII
168	H-DPro	Phe	Arg $NH(CH_2)_7CO$-Gly Gly Arg-NH_2	829	VIII
169	H-DPro	Phe	Arg $NH(CH_2)_7CO$-Gly Arg-NH_2	772	VIII
170	H-DPro	Phe	Arg $NH(CH_2)_7CO$-Gly Gly-Gly Arg-NH_2	886	VIII
171	H-DPro	Phe	Arg N[^{n}Hex]$_2$	586	VIII
172	H-DPro	Cha	Arg $NHCH_2Ch$	520	VIII
173	H-DPro	αNal	Arg $NHCH_2Ch$	564	VIII
174	H-DPro	βNal	Arg $NHCH_2Ch$	564	VIII
175	H-DPro	His	Arg $NHCH_2Ch$	504	VIII
176	H-DPro(4Me)Phe		Arg $NHCH_2Ch$	528	VIII
177	H-DPro	Phe	Nar $NHCH_2Ch$	500	VIII
178	H-DPic	Phe	Arg $NHCH_2Ch$	528	VIII
179	H-DTic	Phe	Arg $NHCH_2Ch$	576	VIII
180	H-DThi	Phe	Arg $NHCH_2Ch$	570	VIII
181	^{n}Bu DPro	Phe	Arg $NHCH_2Ch$	570	VIII

* Satisfactory amino acid analysis obtained

TABLE 8

Ketones

			[M+H]$^+$	Class
49	H-DPro Phe Arg	CH_3	417	I
48	H-DPro Phe Arg	$(CF_2)_2CF_3$	**	I
53	H-DPro Phe Arg	$CH_2CH[^nHex]_2$	***	I

** Molecular ion not detected

TABLE 9

α-Ketoamides

			[M+H]$^+$	Class
11	H-DPro Phe DL Lys	$CON[^nBu]_2$	530	XI
50	H-DPro Cha DL Arg	$CON[^nBu]_2$	***	XI

*** [M+H]$^+$ not available

EXAMPLE I

5 Me-DPhe-Cha-Arg-H

The synthesis of 5 was carried out according to Scheme I. Arabic numerals underlined e.g. 1 refer to structures in these schemes. Roman numerals in parentheses e.g. (i) refer to reaction steps.

(i) Isobutyl chloroformate (10.2 mmol) was added to a solution of Boc-Arg(Z_2)OH (9.23 mmol) and N-methylmorpholine (11.08 mmol) in dry THF (25 cm^3) at -20°C. After 20 mins the solid was filtered off and the filtrate added to a solution of sodium borohydride (10.3 mmol) in water (10 cm^3) at 0°C. After 3 hours 0.3 M $KHSO_4$ was added, the crude product extracted with EtOAc and purified by flash chromatography on silica with EtOAc - petrol (4:6). The alcohol 1 was isolated as a white solid (97%).

(ii) The Boc group of 1 (4.75 mmol) was removed with sat. HCl/Dioxan and the product acylated with Boc-Cha-ONSu (9.5 mmol) in CH_2Cl_2 (20 cm^3) at 0°C in the presence of N-methylmorpholine. After two hours the reaction was worked up using standard procedures and the crude product purified by flash chromatography on silica with EtOAc - petrol (4:6). The pure alcohol 2 was isolated as a colourless oil (90%).

(iii) The Boc group of 2 (4.27 mmol) was removed with sat. HCl/Dioxan and the product reacted with Z(NMe)DPhe-OH (5.12 mmol) in the presence of HOBt (10.2 mmol), water soluble carbodiimide (6.1 mmol) and N-methylmorpholine in DMF (20 cm^3) at 0°C. After 18 hours the reaction was worked up using standard procedures and the product purified by flash chromatography on silica with EtOAc - petrol (1:1). The pure alcohol 3 was isolated as a colourless oil (52%).

(iv) The alcohol 3 (2.22 mmol) was dissolved in CH_2Cl_2/AcOH (30:1) and Dess-Martin Periodinane (4.5 mmol) added. After 2½ hours at room temperature the reaction mixture was diluted with EtOAc and poured into a solution of sodium thiosulphate (32 mmol) and sat. $NaHCO_3$. The crude product was purified by flash chromatography on silica with EtOAc-petrol (3:7). The pure aldehyde 4 was isolated as a colourless oil (75%).

(v) The aldehyde 4 (1.65 mmol) was dissolved in MeOH/H_2O/AcOH (90:9:1, 50 cm^3) and hydrogenated over 5% Pd/C. The crude material was purified by mplc on *Vydac C_{18} (15-25 μ) using MeCN/H_2O/TFA to give pure 5 (CH-851) as a white solid (780 mg). Tlc, EtOAc-Py-AcOH-H_2O (30:20:6:11), R_F 0.66 on silica. After hydrolysis at 110°C/22 hrs with 6N HCl peptide content based on Cha was 40%. FAB mass spec $[M+H]^+ = 473$ (Calc. $^m/z = 472$).

EXAMPLE II

11 H-DPro-Phe-Lys-CON^{n}Bu$_2$ (see Scheme II)

(i) TcbocONSu (14.8 mmol) was added to a solution of H-Lys(Z)-OMe. HCl (12.2 mmol) and triethylamine (14.8 mmol) in CH_2Cl_2 (50 cm^3). After 3 hours at room temperature the reaction was worked up using standard procedures and the product purified by flash chromatography on silica using EtOAc - petrol (7:13). The pure ester 6 was isolated as a colourless oil (100%).

(ii) Diisobutylaluminium hydride (1.5 M solution in toluene, 50 mmol) was added to a solution of 6 (12.2 mmol) in dry toluene (100 cm^3) at -78°C over a period of 20 minutes. After a further 15 minutes methanol (10 cm^3) was added followed by a saturated solution of Rochelle's salt (100 cm^3). After 2½ hours the reaction was worked up using standard procedures and the product purified by flash chromatography on silica using EtOAc - petrol (3:7). The pure aldehyde 7 was isolated as a colourless oil (49%).

* Trade name

(iii) Potassium cyanide (18 mmol) and 1 M hydrochloric acid (30 cm^3) were added to a solution of 7 (5.98 mmol) in ethyl acetate (30 cm^3). After 18 hours at room temperature the reaction was worked up using standard procedures and the product purified by flash chromatography on silica using EtOAc - petrol (4:6). The pure cyanohydrin 8 was isolated as a colourless oil (88%).

(iv) A 4 M solution of HCl in dioxan (50 cm^3) was added to a solution of 8 (5.28 mmol) in dry methanol (15 cm^3) at O°C. After 18 hours at room temperature an ice/water mixture (15 cm^3) was added. After 3 days at 4°C solid $KHCO_3$ was added. The reaction was worked up using standard procedures and the product purified by flash chromatography on silica using EtOAc - petrol (11:9). The pure ester 8b was isolated as a yellow oil (59%).

(v) Activated zinc dust was added in small portions to a solution of 8b (3.1 mmol) in $AcOH/H_2O$ (9:1, 25 cm^3). After 1½ hours at room temperature the zinc was filtered off, the filtrate evaporated in vacuo and the residue was taken up in EtOAc. This solution was washed with sat. $NaHCO_3$, water, brine, dried (Na_2SO_4) and evaporated in vacuo. The amine 9 was isolated as a colourless oil (85%).

(vi) The amine 9 (2.63 mmol) was acylated with Boc-Phe-ONSu (3.04 mmol) in CH_2Cl_2 (30 cm^3) at O°C in the presence of N-methyl morpholine. After 3 hours the reaction was worked using standard procedures and the crude product purified by flash chromatography on silica with EtOAc/Pet Ether (6:4). The pure ester 10a was isolated as a colourless oil (92%).

(vii) The Boc group of 10a (2.41 mmol) was removed using sat. HCl/Dioxan and the product acylated with Boc-DPro-ONSu (2.92 mmol) in CH_2Cl_2 (30 cm^3) at O°C in the presence of N-methyl-morpholine. After 3 hours the reaction was worked up using standard procedures and the product purified by flash chromatography on silica using EtOAc/Petrol (3:1). The pure ester 10b was isolated as a colourless oil (64%).

(viii) Lithium hydroxide (1.6 mmol) and water (3 cm^3) were added to a solution of 10b (1.54 mmol) in THF (30 cm^3). After 4 hours at room temperature the THF was removed in vacuo, the pH of the residue adjusted to pH 4 with 1 M citric acid and extracted with $CHCl_3$. The organic extracts were washed with brine, dried (Na_2SO_4) and evaporated in vacuo. The pure acid 10c was isolated as a colourless oil (70%).

(ix) Pentafluorophenol (1.3 mmol) and water soluble carbodiimide (1.3 mmol) were added to a solution of 10c (1.07 mmol) in CH_2Cl_2 (20 cm^3) at O°C. After 2½ hours dibutylamine (2.1 mmol) was added to this solution at O°C and the pH adjusted to pH 9 with DIEA. After 18 hours at room temperature the reaction was worked up using standard procedures and the product purified by flash chromatography on silica using EtOAc/Petrol (7:3). The pure amide 10d was isolated as a colourless oil (48%).

(x) Dess-Martin Periodinane (0.97 mmol) was added to a solution of 10d (0.52 mmol) in CH_2Cl_2 (100:1, 40 cm^3). After 2 hours at room temperature further Dess-Martin Periodinane (0.52 mmol) was added. After a further 3 hours the reaction mixture was diluted with EtOAc and poured into a solution of sodium thiosulphate (7.3 mmol) in water and sat. $NaHCO_3$ were added. The crude product was purified by flash chromatography on silica with EtOAc/petrol (9:11). The pure keto amide 10e was isolated as a colourless oil (57%).

(xi) The Boc group of 10e (0.24 mmol) was removed using sat. HCl/Dioxan. The resultant product was dissolved in AcOH/H_2O (9:1) and hydrogenated over 5% Pd/C. The crude material was purified by mplc on *Vydac C_{18} (15 - 25 μ) using MeCN/H_2O/TFA to give 11 (CH-1463 89.7 mg). Hplc, *Novapak C_{18}, 4 μ (8 x 100 mm), linear gradient 20 → 80% 0.1% TFA/MeCN into 0.1% TFA/H_2O over 25 min at 1.5 ml min^{-1} indicates the presence of two epimers D-Arg (40%) at 11.2 min and L-Arg (60%) at 12.6 min. After hydrolysis at 110°C/22 h with 6N HCl, amino acid analysis Phe 0.93, Pro 1.07.

EXAMPLE III

17 H-DPro-Phe-Arg-$CH_2S(CH_2)_4CH_3$ (see Scheme III)

(i) Boc-Arg(Z_2)OH (46.1 mmol) was dissolved in dry THF (200 cm^3). N-methylmorpholine (50.85 mmol) and isobutyl chloroformate (50.73 mmol) were added at -20°C. After 20 min. this mixture was added to a solution of diazomethane (0.1 mole) in Et_2O at -5°C. After 2 hours the diazoketone 12 was isolated as a yellow solid.

(ii) The diazoketone 12 (46.1 mmol) in dry THF was treated with HBr (69.15 mmol) in EtOAc at -20°C followed by addition of sat. $NaHCO_3$ after 45 mins. The crude product was extracted with EtOAc and crystallised from EtOH to give pure Boc-Arg(Z_2)CH_2Br, 13, (85%).

(iii) 1-Pentanethiol (1.27 mmol) in dry DMF (5 cm^3) was treated with sodium hydride (1.4 mmol). After 30 mins Boc-Arg(Z_2)CH_2Br 13 (1.27 mmol) was added-40°C for 20 mins and -5°C for 2½ hours. After addition of 0.3 M $KHSO_4$ and extraction of the crude product with EtOAc, flash chromatograpy on silica with EtOAc - petrol (15:85) yielded the pure thiomethylene compound 14 as a colourless oil (74%).

* Trade name

(iv) The Boc protecting group of 14 (0.94 mmol) was removed using sat. HCl/Dioxan and the resulting product was acylated with Boc-Phe-OPfp (1.13 mmol) in CH_2Cl_2 at O°C in the presence of DIEA. The crude product was purified by flash chromatography on silica with EtOAc - petrol (3:7) yielding the pure thiomethylene analogue 15 as a colourless oil (55%).

(v) The Boc protecting group of 15 (0.52 mmol) was removed using sat. HCl/Dioxan. The resulting product was dissolved in DMF and treated with Boc-DPro-OH (0.63 mmol) in the presence of HOBt (1.05 mmol), water soluble carbodiimide (0.76 mmol) and N-methylmorpholine. After a standard work-up the crude material was purified by flash chromatography on silica with EtOAc - petrol (4:6) yielding the pure thiomethylene compound 16 as a colourless oil (74%).

(vi) The Boc protecting group of 16 (0.38 mmol) was removed using sat. HCl/Dioxan. The resultant product was dissolved in AcOH/H_2O (9:1) and hydrogenated over 5% Pd/C. The crude material was purified by mplc on *Vydac C_{18} (15-25 μ), using MeCN/H_2O/TFA to give pure 17 (CH-574, 41 mg). Hplc, *Novapak C_{18}, 4μ (8 x 100 mm), linear gradient 20 → 80% 0.1% TFA/MeCN into 0.1% TFA/H_2O over 25 min at 1.5 ml min^{-1} indicates the presence of two epimers D-Arg (<5%) at 9.8 min and L-Arg (>95%) at 11.1 min. After hydrolysis at 150°C/1.5 h with 6N HCl, amino acid analysis Phe, 0.80; Pro, 1.00.

All analogues in Table 2 were synthesised by the described method. 61 was synthesised by the oxidation of 60 with meta-chloroperoxybenzoic acid.

* Trade name

EXAMPLE IV

23 H-DPro-Phe-Arg-$CH_2OCH_2(CF_2)_3CHCF_2$ (see Scheme IV)

(i) IH, IH, 5H-Octafluoro-1-pentanol (2.45 mmol) in dry DMF (8 cm^3) was treated with sodium hydride (1.83 mmol). After 30 mins the bromoketone 13 (1.65 mmol) was added at -40°C and left at this temperature for 30 mins and -5°C for 2½ hours. Addition of 0.3 M $KHSO_4$ and extraction with EtOAc gave the crude product which was purified by flash chromatography on silica using EtOAc - petrol (15:85). The pure fluoroether 18 was isolated as a colourless oil (69%).

(ii) The fluoroether 18 (1.13 mmol) was dissolved in MeOH (40 cm^3), sodium borohydride (1.18 mmol) was added to this solution at 0°C. After 15 min 0.3 M $KHSO_4$ was added and the mixture extracted with EtOAc giving the pure compound 19 as a colourless oil (88%).

(iii) The Boc protecting group of 19 (1.0 mmol) was removed with sat. HCl/Dioxan. The resulting product was dissolved in CH_2Cl_2 and acylated with Boc-Phe-OPfp (1.2 mmol) in the presence of DIEA at 0°C. After a standard work up the crude product was purified by flash chromatography on silica with EtOAc - petrol (3:7) yielding the pure product 20 as a colourless oil (55%).

(iv) 20 (0.55 mmol) was deprotected with sat. HCl/Dioxan and acylated with Boc-DPro-OPfp (1.63 mmol) in CH_2Cl_2 at 0°C in the presence of DIEA. After a standard work up the crude product was purified by flash chromatography on silica with EtOAc - petrol (4:6) yielding the pure product 21 as a colourless oil (48%).

(v) 21 (0.24 mmol) was dissolved in CH_2Cl_2/AcOH (30:1) and Dess-Martin Periodinane (0.48 mmol) was added. After 2 hours at room temperature the reaction mixture was diluted with EtOAc and poured into a solution of sodium thiosulphate (3.5 mmol) in water and sat. $NaHCO_3$. The crude product was purified by flash chromatography on silica with EtoAc - petrol (7:13) yielding the pure fluoroether 22 as a colourless oil (64%).

(vi) The fluoroether 22 (0.16 mmol) was deprotected and purified as described in Example III (vi). Pure 23 (CH-619) was isolated as a white solid (50.9 mg). Hplc, *Novapak C_{18} 4μ (8 x 100 mm), linear gradient 20 → 80% 0.1% TFA/MeCN into 0.1% TFA/H_2O over 25 mins at 1.5 ml min^{-1} indicated a single product (T_R = 11.5 min). After hydrolysis at 150°C/1.5 hr with 6N HCl, amino acid analysis Phe, 1.00; Pro, 1.2.

All analogues in Table 3 were synthesised by the described method except 54 and 55 which were synthesised by methods outlined in Schemes XIII and XIV respectively.

EXAMPLE V

31 H-DPro-Phe-Arg-$CH_2N[(CH_2)_5CH_3]_2$ (see Scheme V)
DL

(i) Boc-Arg(Z_2)OH (18.5 mmol) was dissolved in CH_2Cl_2 (50 cm^3). To this solution at 0°C was added trichloroethanol (20.35 mmol), water soluble carbodiimide (22.2 mmol) and dimethylaminopyridine (0.93 mmol). After 3 hours the reaction was worked up using standard procedures giving the pure trichloroethyl derivative 24 as a colourless oil (100%).

* Trade name

(ii) 24 (18.1 mmol) was deprotected with sat. HCl/Dioxan and acylated with Boc-Phe-ONSu (27.2 mmol) in CH_2Cl_2 at 0°C in the presence of N-methylmorpholine. After 3 hours the reaction mixture was worked up using standard procedures and the crude product was purified by flash chromatography on silica with EtOAc - petrol (2:8) yielding the pure product 25 as a white solid (97%).

(iii) 25 (17.4 mmol) was deprotected with sat. HCl/Dioxan and acylated with Boc-DPro-ONSu (26.2 mmol) in CH_2Cl_2 at 0°C in the presence of N-methylmorpholine. After 3 hours the reaction was worked up using standard procedures and the crude product purified by flash chromatography on silica with EtOAc - petrol (35:65) giving the pure protected tripeptide 26 as a colourless oil (94%).

(iv) Activated zinc powder was added to a solution of 26 (16.47 mmol) in glacial acetic acid. After 3 hours at room temperature the zinc was filtered off, the filtrate evaporated and the crude product purified by flash chromatography on silica with EtOAc - petrol - AcOH (74:25:1) giving the pure tripeptide 27 as a white solid (91%).

(v) The protected tripeptide 27 (15 mmol) was dissolved in dry THF (40 cm^3), N-methylmorpholine (18 mmol) and isobutyl-chloroformate (16.5 mmol) were added at -20°C. After 20 mins the mixture was added to a solution of diazomethane (35 mmol) in Et_2O at -5°C. After 2½ hours the diazoketone 28 was isolated as a yellow oil.

(vi) The diazoketone 28 (15 mmol) in dry THF was treated with HBr (22.5 mmol) in EtOAc at -20°C followed by addition of sat. $NaHCO_3$ after 45 mins. The crude product was extracted with EtOAc and purified by flash chromatography on silica with EtOAc - petrol (1:1). The pure bromoketone 29 was isolated as a white solid (72%).

(vii) Dihexylamine (1.25 mmol) and $NaHCO_3$ (0.8 mmol) were added to a solution of bromoketone 29 (0.23 mmol) in dry THF (5 cm^3). After 18 hrs at room temperature 0.3 M $KHSO_4$ was added to the reaction mixture and the crude product was purified by flash chromatography on silica with EtOAc - petrol (35:65). The protected aminomethylene ketone 30 was isolated as a yellow oil (54%).

(viii) The aminomethylene ketone 30 (0.12 mmol) was deprotected and purified as described in Example III (vi). Pure 31 (CH-694) was isolated as a white solid (41 mg). Hplc, linear gradient 20 → 80% 0.1% TFA/MeCN into 0.1% TFA/H_2O over 25 mins at 1.5 ml min^{-1} indicated the presence of two epimers D-Arg (50%) at 11.2 min and L-Arg (50%) at 12.5 mins. After hydrolysis at 110°C/22 hrs with 6N HCl, amino acid analysis Phe, 0.91; Pro, 1.09.

All analogues in Table 4 were synthesised by the described method. The required amines were synthesised by standard synthetic methods such as reductive amination and the Curtius rearrangement.

EXAMPLE VI

40 H-DPro-Phe-Arg$^{K}_{DL}$Gly-Pro-NHEt (see Scheme VI)

(i) $H_2C(CO_2Tce)_2$ (6.81 mmol) was treated with sodium hydride (5.67 mmol) in dry THF (30 cm^3). After 45 mins the bromoketone 13 (4.52 mmol) was added at -5°C. After 2½ hours 0.3 M $KHSO_4$ was added, the crude product extracted with EtOAc and purified by flash chromatography on silica with EtOAc - petrol (2:8). The pure Boc-Arg(Z_2)$CH_2CH(CO_2Tce)_2$ 32 was isolated as a colourless oil (83%).

(ii) Activated zinc was added to a solution of 32 (3.65 mmol) in glacial acetic acid. After 2½ hours at room temperature the zinc was filtered off, the filtrate evaporated and the diacid 33 isolated.

(iii) A solution of the diacid 33 in toluene was heated at reflux for 45 mins. The solvent was evaporated and the crude product purified by flash chromatography on silica with EtOAc - petrol - AcOH (60:39:1). The Boc-Arg(Z_2)^{K}Gly-OH 34 was isolated as a colourless oil (70% from 32).

(iv) Trichloroethanol (2.82 mmol), water soluble carbodiimide (2.81 mmol) and dimethylaminopyridine (0.117 mmol) were added to a solution of 34 (2.34 mmol) in CH_2Cl_2 (50 cm^3) at 0°C. After 2½ hours the reaction was worked up using standard procedures and the crude product purified by flash chromatography on silica with EtOAc - petrol (85:15). The trichloroethyl derivative 35 was isolated as a colourless oil (83%).

(v) The Boc protecting group of 35 (1.85 mmol) was removed using sat. HCl/Dioxan and the resulting product acylated with Boc-Phe-OPfp (6.04 mmol) in CH_2C_2 in the presence of DIEA. After 2 hours the reaction was worked up using standard procedures and the crude product purified by flash chromatography on silica with EtOAc - petrol (2:8). The pure product 36 was isolated as a colourless oil (85%).

(vi) The Boc protecting group of 36 (1.58 mmol) was removed using sat. HCl/Dioxan and the resulting product acylated with Boc-DPro-OPfp (5.12 mmol) in CH_2Cl_2 in the presence of DIEA. After 2 hours the reaction was worked up using standard procedures and the crude product purified by flash chromatography on silica with EtOAc - petrol (35:65). The pure product 32 was isolated as a colourless oil (79%).

(vii) Activated zinc dust was added to a solution of 37 (1.13 mmol) in glacial acetic acid. After 2½ hours at room temperature the zinc was filtered off, the filtrate evaporated and the crude product purified by flash chromatography on silica with EtOAc - petrol - AcOH (70:29:1). The product 38 was isolated as a colourless oil (76%).

(viii) The protected keto isostere 38 (0.26 mmol) was converted to its Pfp ester by treatment with Pfp-OH (0.29 mmol) and water soluble carbodiimide (0.31 mmol) in CH_2Cl_2 (8 cm^3) at 0°C for 2½ hours. This Pfp ester was coupled at 0°C to H-Pro-NHEt . HCl salt (0.78 mmol) in the presence of DIEA. After 18 hours the reaction was worked up using standard procedures and the product purified by flash chromatography on silica with $CHCl_3$-MeOH-AcOH (97:2:1). The product 39 was isolated as a colourless oil (91%).

(ix) The protected keto isostere containing analogue 39 (0.23 mmol) was deprotected as described in Example III (vi). Pure 40 (CH-595) was isolated as a white solid (40 mg). Hplc, linear gradient 10 → 50% 0.1% TFA/MeCN into 0.1% TFA/H_2O over 25 mins at 1.5 ml min^{-1} indicated the presence of two epimers D-Arg (46%) at 10.1 min and L-Arg (54%) at 11.7 min. After hydrolysis at 150°C/1.5 hrs with 6N HCl amino acid analysis Phe, 0.91; Pro, 1.09.

All analogues in Table 5 were synthesised by the described method.

EXAMPLE VII

45 H-DPro-Phe-Arg-Chg-NH_2 (see Scheme VII)

(i) Boc-Phg-OH (19.9 mmol) was dissolved in AcOH/H_2O (9:1, 100 cm^3) and hydrogenated over Rh/C at 60 p.s.i. for 3 days. The catalyst was filtered off and the solvent removed to give Boc-Chg-OH 41 (100%).

(ii) Water soluble carbodiimide (4.1 mmol) and HOBt (4.3 mmol) were added to a solution of 41 (3.9 mmol) in CH_2Cl_2/DMF (2:1, 60 cm^3) at room temperature. After 30 mins 35% ammonia solution (0.8 cm^3) was added. After a further 3 hours at room temperature the reaction was worked up using standard procedures and the product recrystallised from EtOH to give the pure amide 42 as a white solid (60%).

(iii) The Boc group of 42 (0.77 mmol) was removed with sat. HCl/Dioxan to give the amide 43 as a white solid (100%).

(iv) The protected tripeptide 27 (0.38 mmol) was dissolved in DMF (5 cm^3). 43 (0.76 mmol), HOBt (0.76 mmol) water soluble carbodiimide (0.46 mmol), and N-methylmorpholine were added at 0°C. After 18 hours at room temperature the reaction was worked up using standard procedures and the product purified by flash chromatography on silica with $CHCl_3$/MeOH (99:1). The pure protected tetrapeptide 44 was isolated as a white solid (69%).

(v) The protected tetrapeptide 44 (0.27 mmol) was deprotected and purified as described in Example III (vi). Pure 45 (CH-640) was isolated as a white solid (58 mg). Hplc, linear gradient 10 → 50% 0.1% TFA/MeCN into 0.1% TFA/H_2O over 25 min at 1.5 ml min^{-1}, single peak detected at 14.2 min. After hydrolysis at 110°C/22 hrs with 6N HCl, amino acid analysis, Arg, 0.96; Phe, 1.00; Pro, 0.95.

All analogues in Table 6 were synthesised by the described method or by other standard peptide coupling methodology. (M. Bodansky & A. Bodansky, The Practice of Peptide Synthesis, Springer-Verlag, 1984)

EXAMPLE VIII

47 H-DPro-Phe-Arg-N[$(CH_2)_5CH_3$]$(CH_2)_3$Ch (see Scheme VIII)

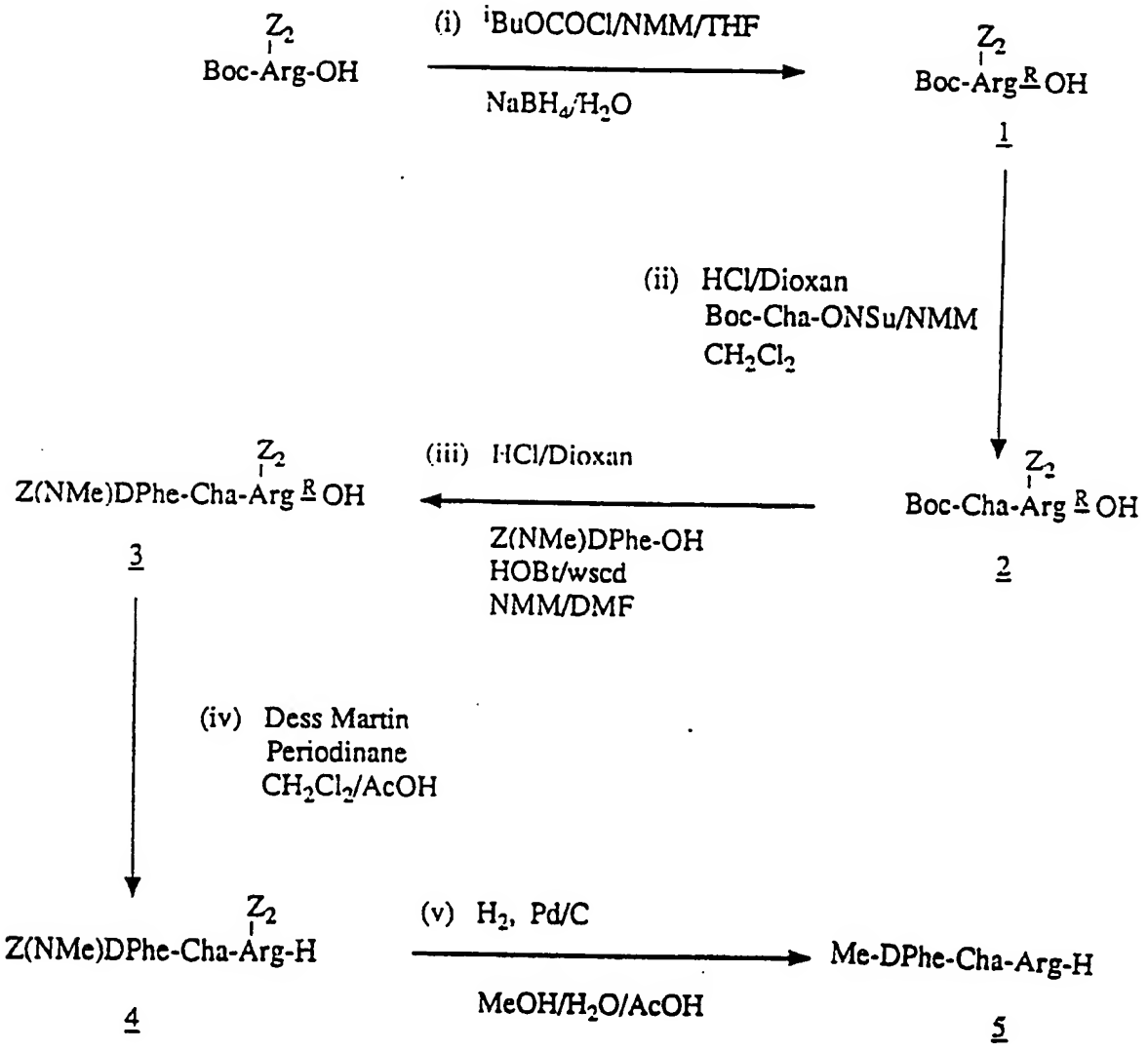
(i) The protected tripeptide 27 (0.32 mmol) was dissolved in DMF (5 cm^3), HN[$(CH_2)_5CH_3$]$(CH_2)_3$Ch (0.96 mmol), HOBt (0.64 mmol), water soluble carbodiimide (0.38 mmol) and N-methylmorpholine were added at O°C. After 18 hours at room temperature the reaction was worked up using standard procedures and the product purified by flash chromatography on silica using EtOAc/Hexane (4:6). The amide 46 was isolated as a colourless oil (32%).

(ii) The protected tripeptide amide 46 (0.1 mmol) was deprotected and purified as described in Example III (vi). Pure 47 (CH-985) was isolated as a white solid (17 mg). Hplc, linear gradient 40 → 90% 0.1% TFA/MeCN into 0.1% TFA/H_2O over 25 min at 1.5 ml min^{-1}, single peak detected at 10.7 min. After hydrolysis at 110°C for 22 hrs with 6N HCl, amino acid analysis Phe, 1.01; Pro, 0.99.

All analogues in Table 7 were synthesised by the described method. The required tripeptide presursors were synthesised in a similar manner to 27 or by standard peptide coupling methodology. (M. Bodansky & A. Bodansky, The Practice of Peptide Synthesis, Springer-Verlag, 1984.) Required amines were either commercially available or synthesised via the Curtius rearrangement. ^{n}Bu-DPro-OH for 181 was synthesised by reductive amination.

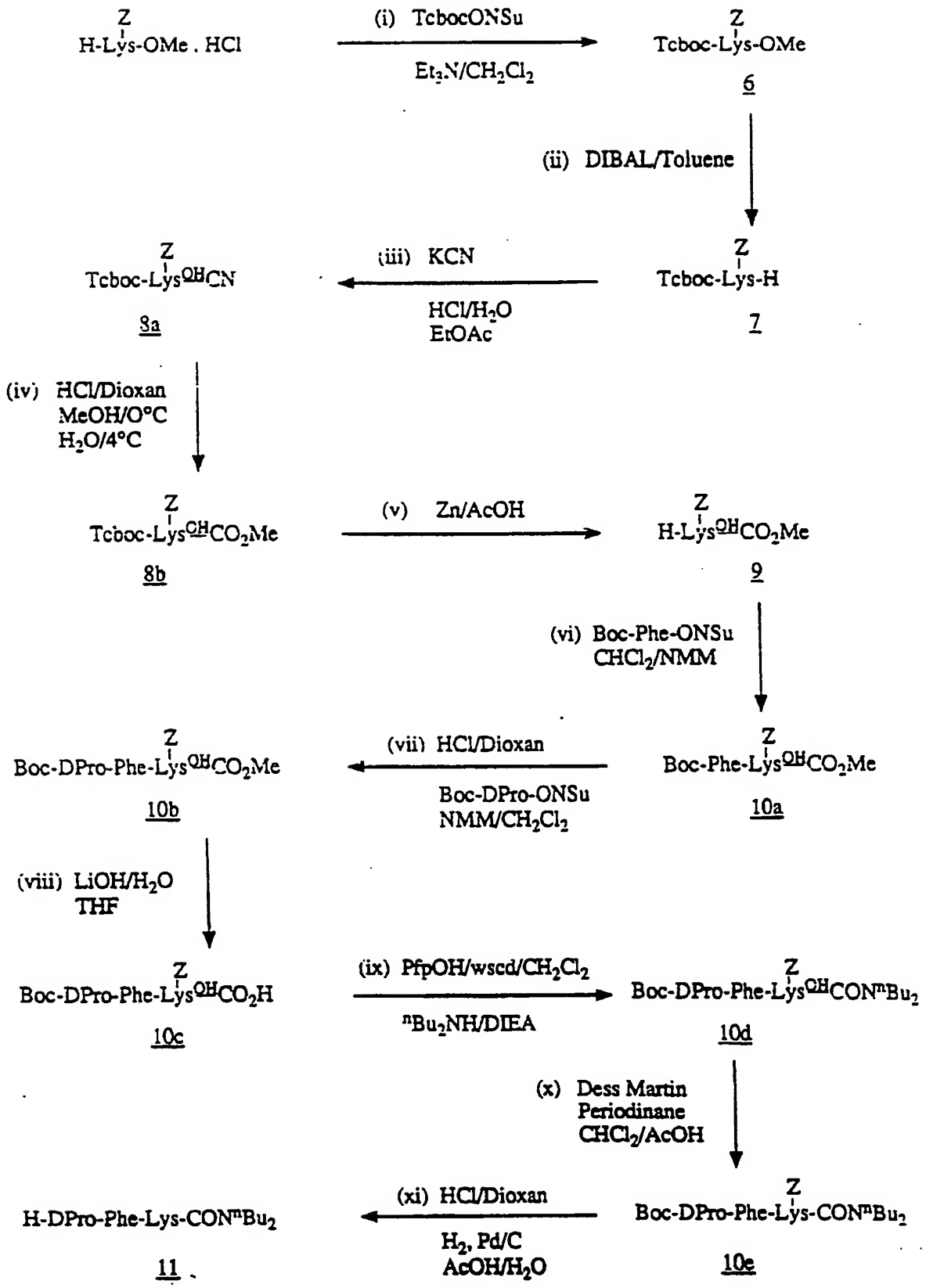
Scheme 1

(Synthesis of compound 5)



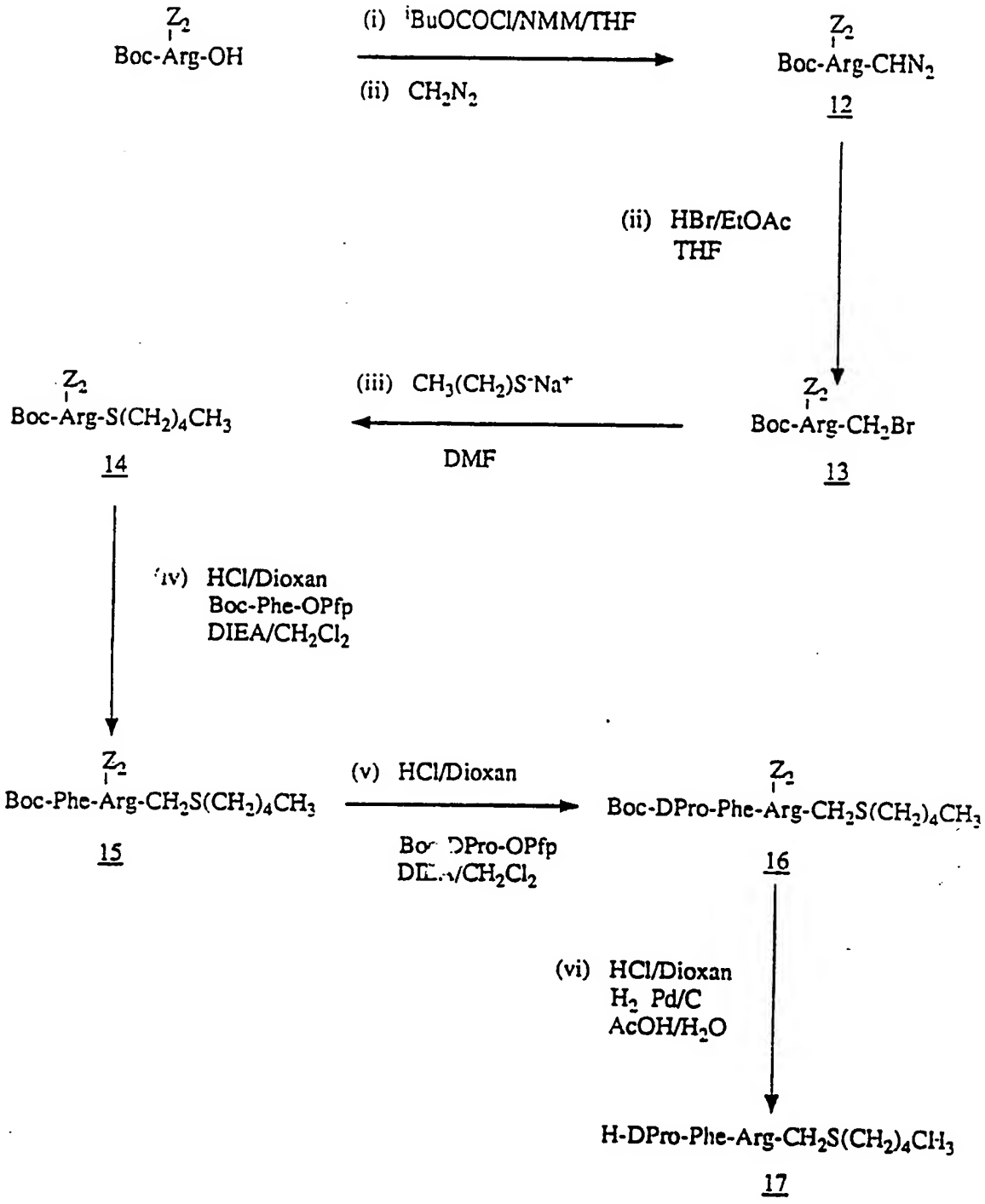
Scheme II

(Synthesis of compound 11)



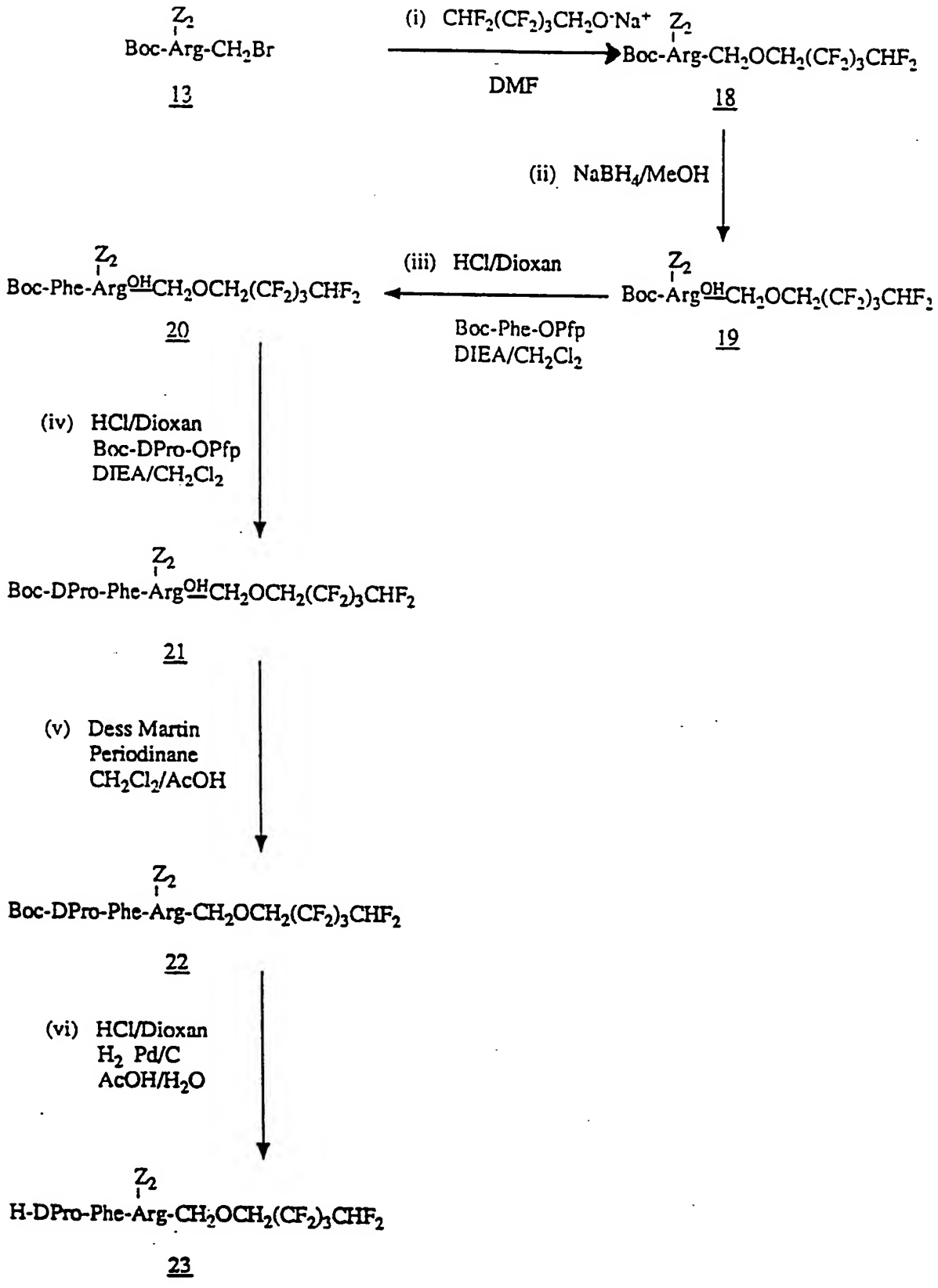
Scheme III

(Synthesis of compound 17)



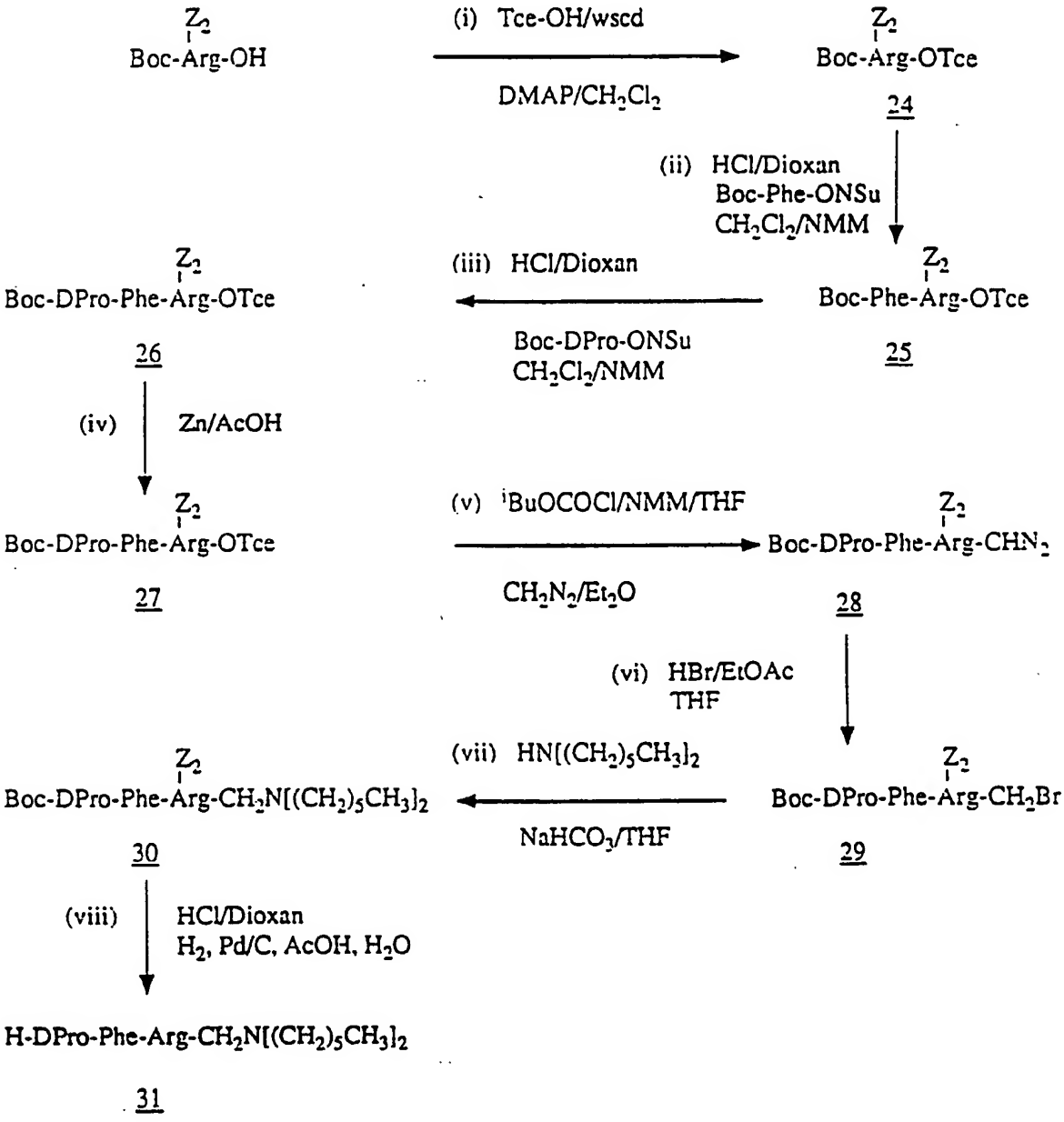
Scheme IV

(Synthesis of compound 23)



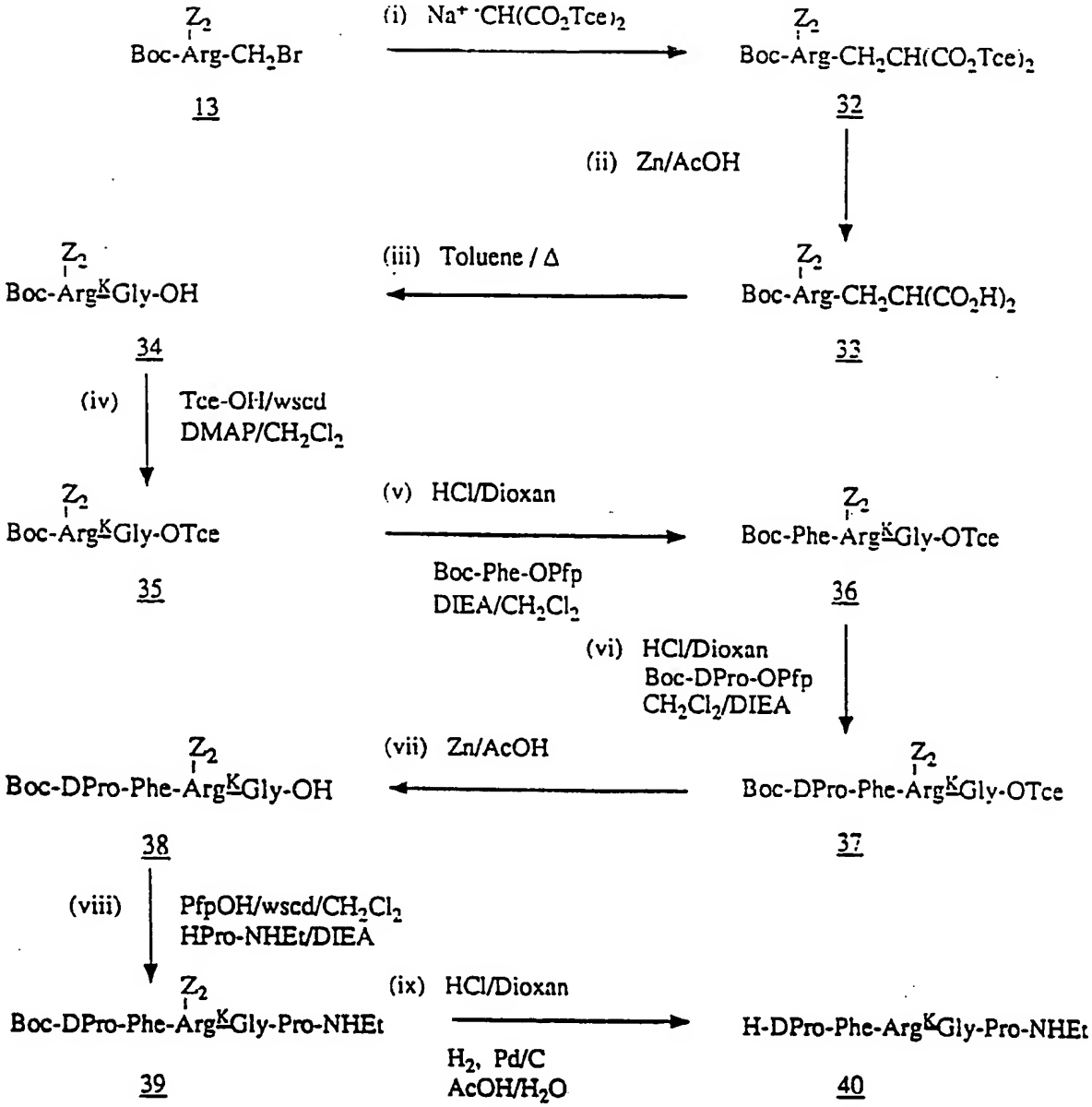
Scheme V

(Synthesis of compound 31)



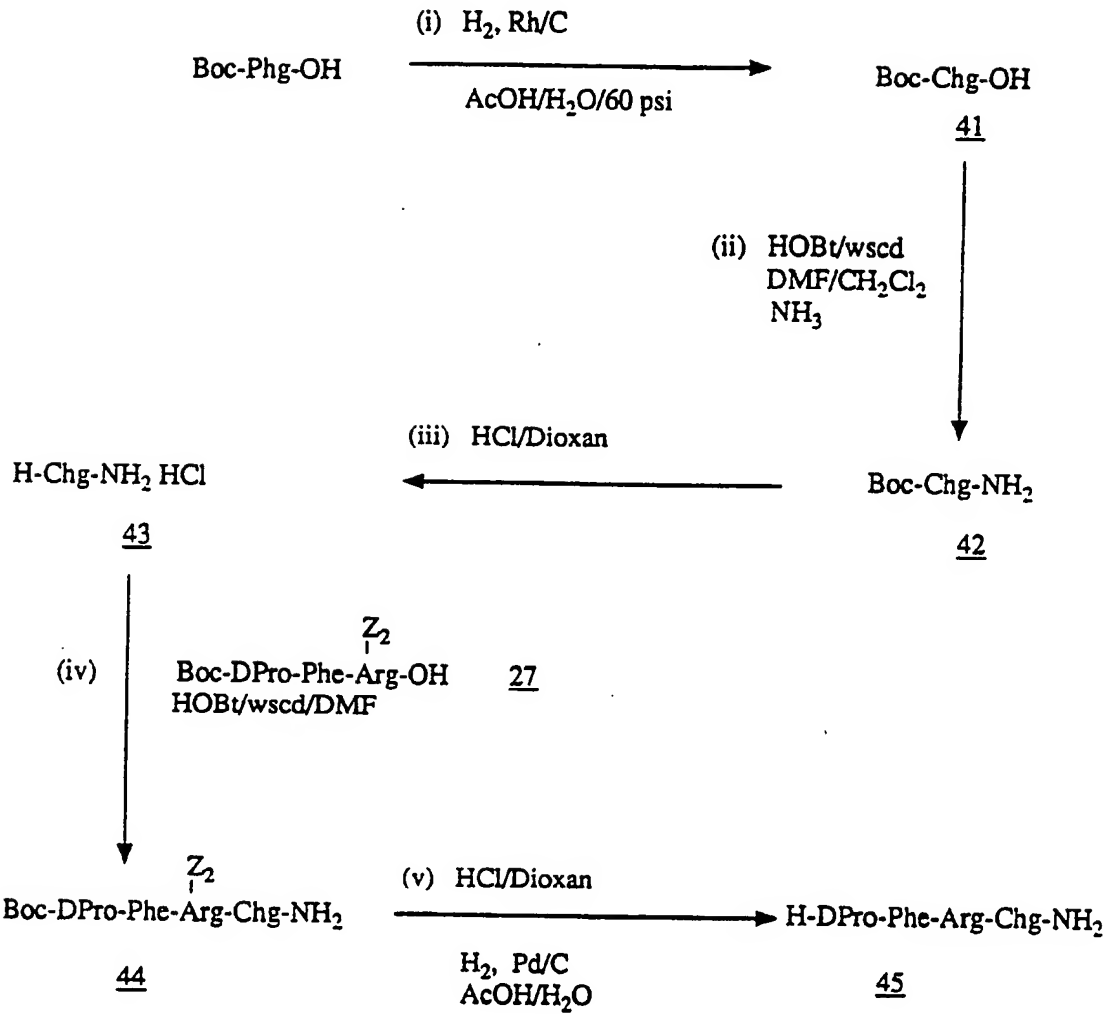
Scheme VI

(Synthesis of compound 40)



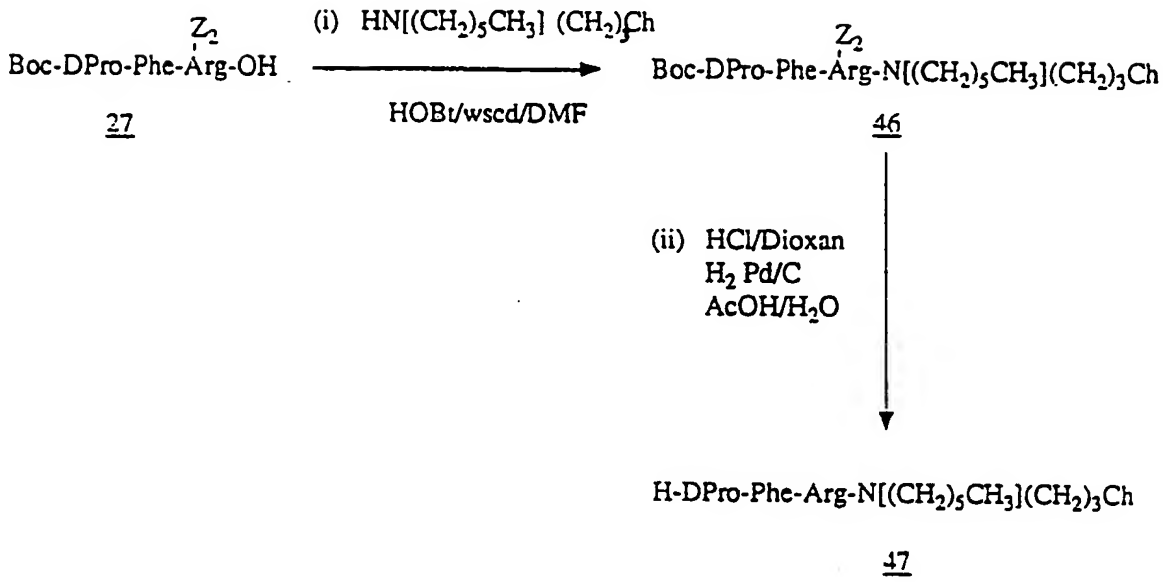
Scheme VII

(Synthesis of compound 45)



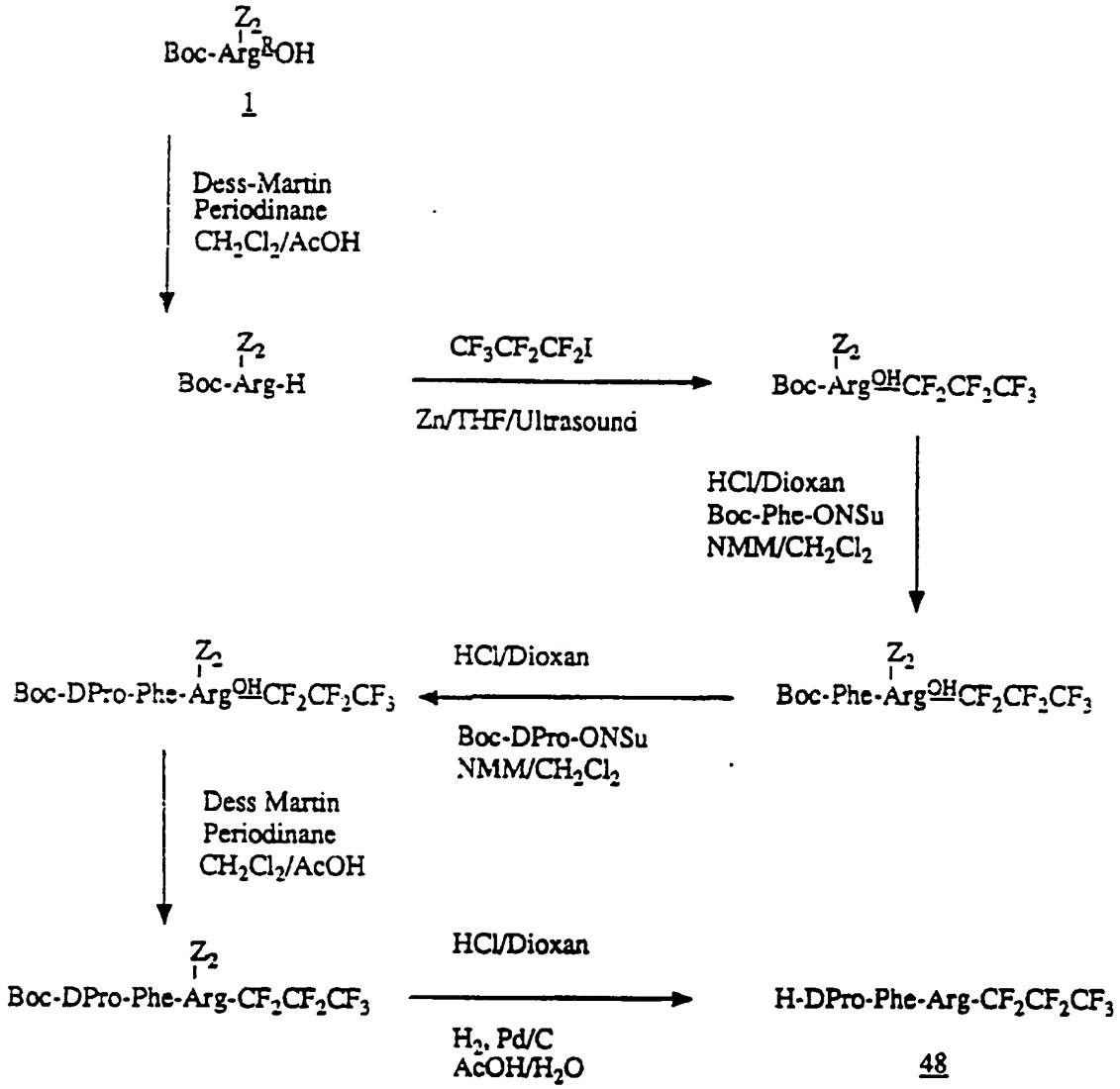
Scheme VIII

(Synthesis of compound 47)



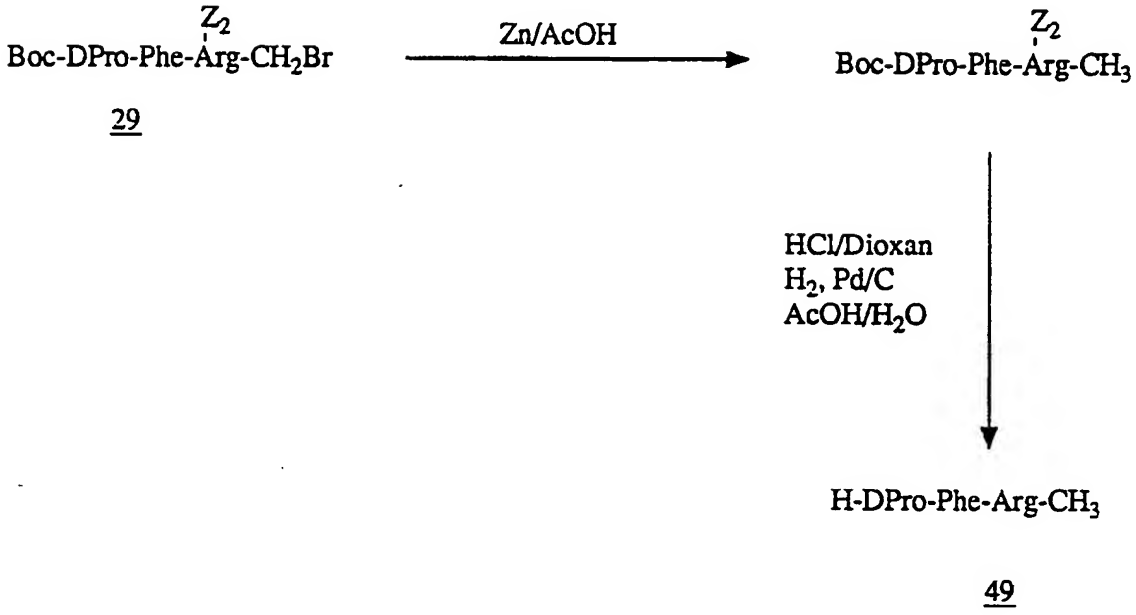
Scheme IX

(Synthesis of compound 48)



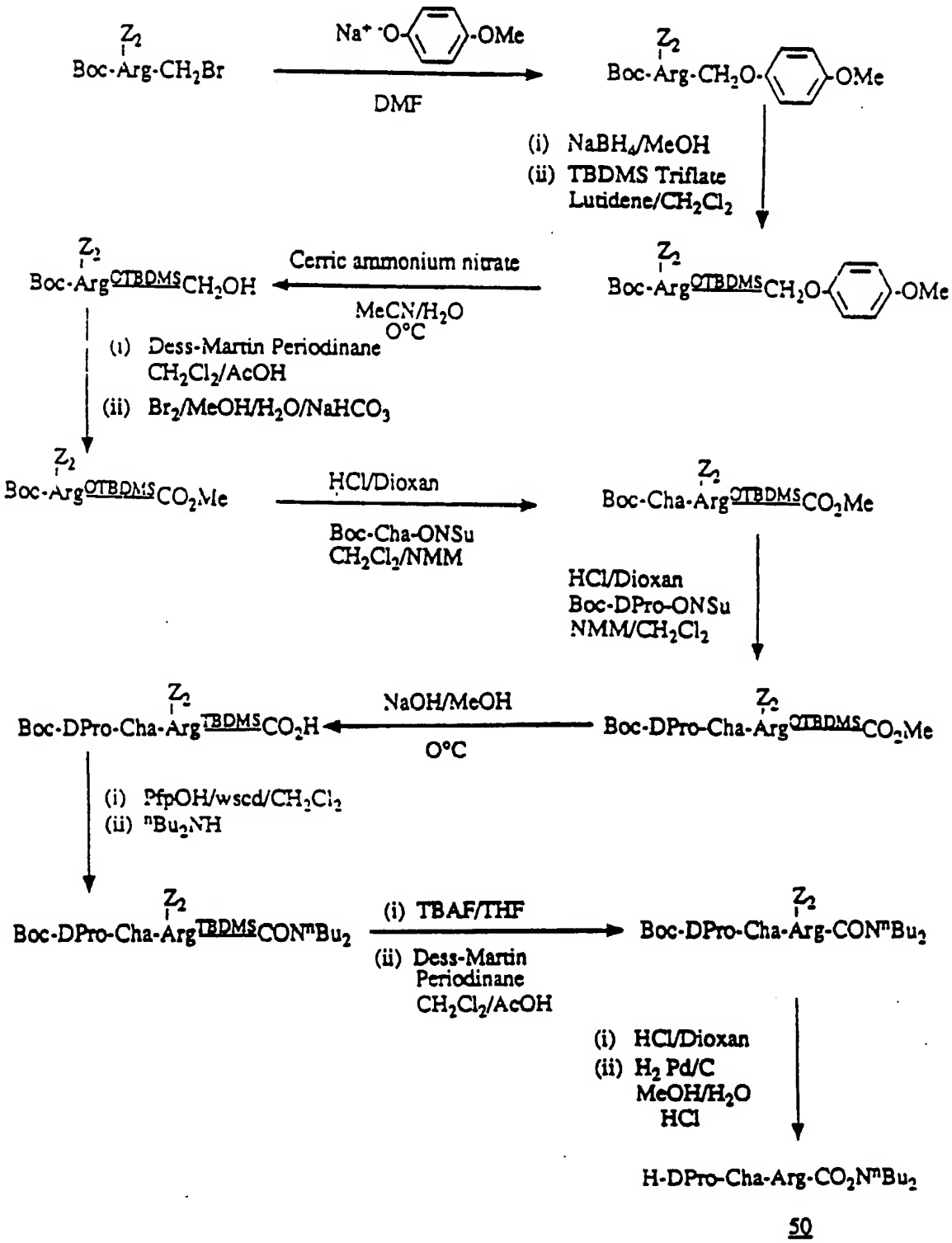
Scheme X

(Synthesis of compound 49)



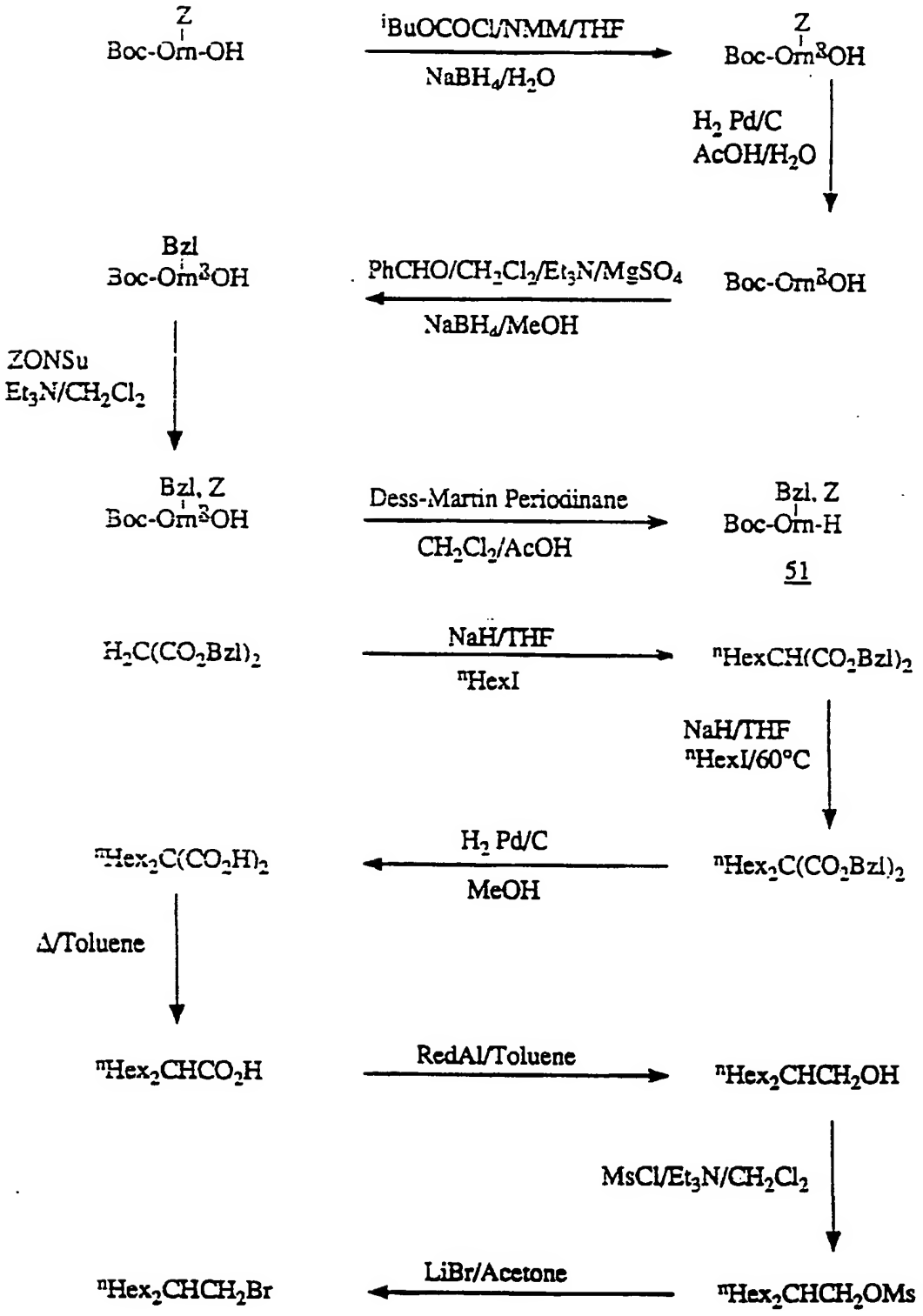
Scheme XI

(Synthesis of compound 50)



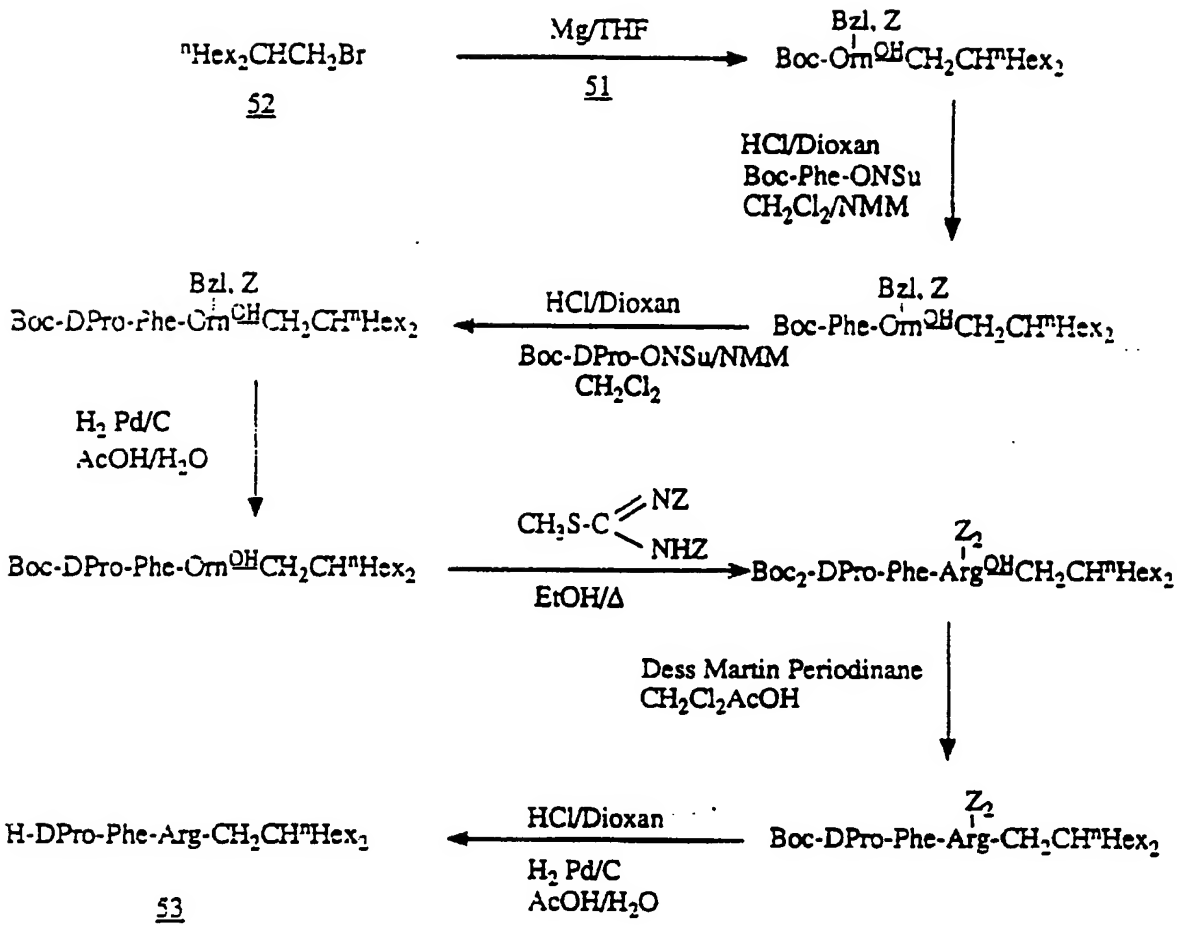
Scheme XII

(Synthesis of compound 53)



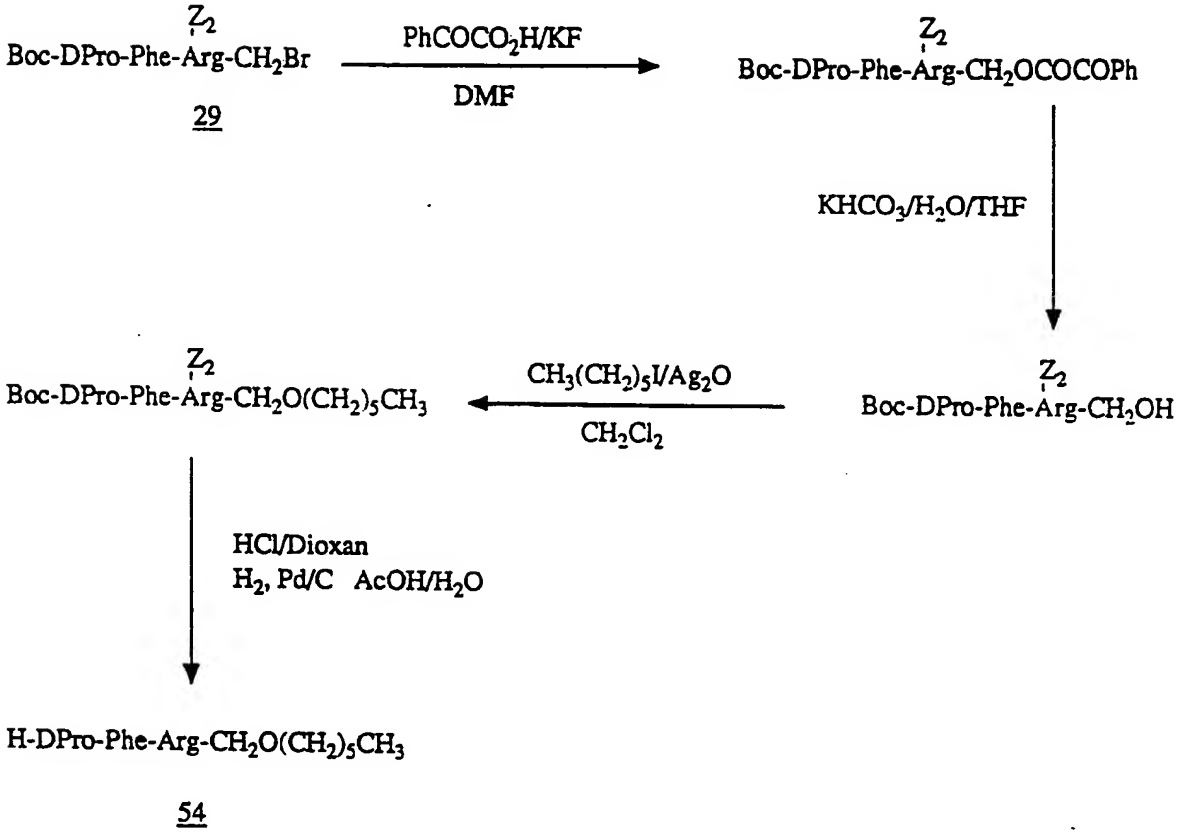
(cont.)

Scheme XII (cont.)



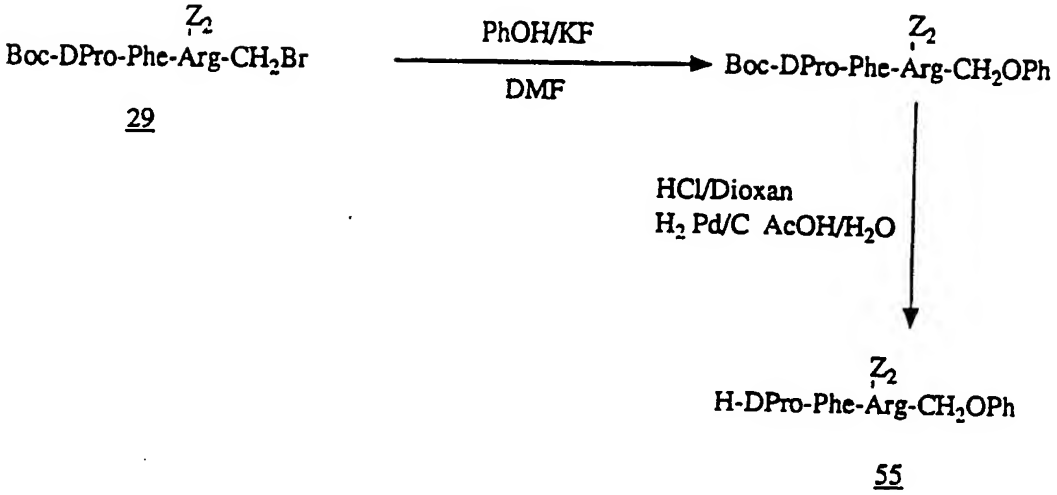
Scheme XIII

(Synthesis of compound 54)



Scheme XIV

(Synthesis of compound 55)



ABBREVIATIONS USED

Abn	3-Azabicyclo[3.2.2]-nonane
Ac	Acetyl
AcOH	Acetic acid
Ada	Adamantylalanine
Aha	2-Aminohexanoic acid (Norleucine)
Boc	tert-Butyloxycarbonyl
Bu	Butyl
Ch	Cyclohexyl
Cha	Cyclohexylalanine
Chg	Cyclohexylglycine
Cpr	Cyclopropyl
DIEA	Diisopropylethylamine
DMAP	4-Dimethylamino-pyridine
DMF	Dimethylformamide
EtOAc	Ethyl acetate
FAB	Fast Atom Bombardment
4-Fph	4-Fluorophenylalanine
Hch	Homocyclohexylalanine
HOBt	1-Hydroxybenzotriazole
hplc	high performance liquid chromatography
Hyp	4-Hydroxyproline
tHyp	trans-4-Hydroxyproline

K	keto isostere -$COCH_2$-
Me	Methyl
MeCN	Acetonitrile
MeOH	Methanol
mplc	medium pressure (preparative) liquid chromatography
Nal	Naphthylalanine
NMM	N-Methylmorpholine
Npg	Neopentylglycine
Oc	Octyl
OH	Hydroxy isostere -CHOH-
ONSu	hydroxysuccinimide
Petrol	Petroleum ether 60 - 80°C
Pfp	Pentafluorophenyl
Phe-4NO_2	4-Nitrophenylalanine
Phg	Phenylglycine
Pic	Pipecolinic acid
Pip	Piperidyl
Py	Pyridine
R	Reduced isostere -CH_2-
Sar	Sarcosine (N-methylglycine)
TBAF	Tetrabutylammonium fluoride
TBDMS	tert-Butyldimethylsilyl
Tcboc	(1-Dimethyl-1-trichloromethyl)ethoxy carbonyl
Tce	2,2,2-Trichloroethyl

Tha	3,3,5-Trimethylhexahydroazepyl
THF	Tetrahydrofuran
Thi	Thienylalanine
Tic	1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid
tlc	thin layer chromatography
wscd	water soluble carbodiimide
Z	Benzyloxycarbonyl
Nor	Norarginine

BIOLOGICAL ACTIVITY; MEDICAL USE

Compounds were tested in vitro for the following activities using standard procedures:

(a) Inhibition of human tissue kallikrein, plasma kallikrein and mast cell tryptase hydrolysing the chromogenic substrates S-2266, S-2302 and S-2266 respectively (method is adapted from that of Johansen, H.T., et al., Int. J. Tiss. Reac., 1986, 8, 185-192). A series of measurements were carried out using a number of different inhibitor concentrations and at least two different substrate concentrations. The inhibitory constant Ki was determined graphically, using a Dixon plot (M. Dixon, Biochem. J., 1953, 55, 170).

(b) Inhibition of kinin release from low and high molecular weight kininogens by tissue and plasma kallikrein respectively. A series of measurements were carried out using two substrate concentrations. The activity is calculated as the amount of kinen released per minute, this being determined by radioimmunoassay using polyclonal antibodies. The inhibitory constant Ki was determined graphically using a Dixon plot.

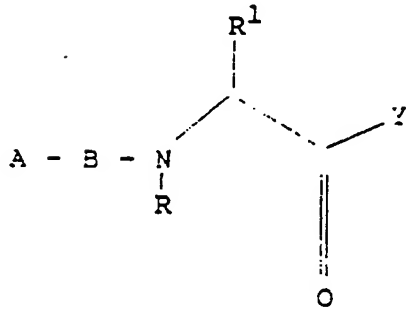
All the examples in Tables 1 - 9 have Ki values in the range 10^{-5} - 10^{-9} M against one or all of the enzymes in the chromogenic assay.

In vivo activity has been tested in well-established pharmacology models of asthma based on the sensitised guinea pig. A selection of these inhibitors representing the different chemical types proved to be highly effective in blocking both the acute phase response and the late phase reaction, their efficacy being comparable or superior to those of the topical steroids and β_2-agonist currently used in asthma therapy.

When the compounds of the present invention are used as a medicine, there are no critical limitations to the administration methods. The present enzyme inhibitor can be formulated by any conventional method in pharmaceutics. For example, the present enzyme inhibitor may be applied in any conventional manner including intravenous injection, intramuscular injection, instillation, oral administration. respiratory inhalation, instillation, rhinenchysis, and external skin treatment. Although there are no critical limitations to the administration dosage, the suitable dosage is 1 to 1000 mg/day/person.

C L A I M S

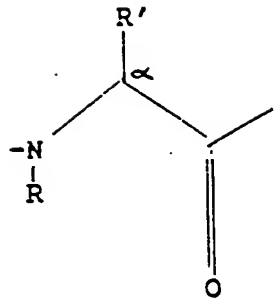
1. Kininogenase inhibitors, optimally not exceeding the size of a hexapeptide, represented by:-



wherein

A and B = amino acyl (including amino acyl analogue) the same or different forming a dipeptide group the amino acid of A carrying a terminal group and being any amino or imino-acid residue (but preferably of D-configuration) and of B being a lipophilic amino-acid residue of D- or L-configuration but not proline or a proline analogue, or a conformational analogue of said dipeptide group wherein the peptide link is replaced by $-CH_2-NH-$ ('reduced'), $-CH(OH)-CH_2-$ ('hydroxy'), $-CO-CH_2-$ ('keto'), $-CH_2-CH_2-$ ('hydrocarbon') or other conformational mimic of the peptide bond

and in:-



the side chain R^1 is that of a basic amino acid or amino acid analogue (preferably of L-configuration) and R is H or lower alkyl (C_1 - C_4) or C^α or the peptide link comprising -N(R)- is replaced leading to a conformational mimic as above

Y = a binding enhancing or carbonyl activating group preferably selected from H (when A or B must be cyclohexylalanine, preferably D if at A or L if at B) or alkyl (C_1 - C_{20}) or fluoroalkyl (C_2 - C_{12}); substituted oxymethylene; thiomethylene; sulphoxy-methylene; sulphonylmethylene; aminomethylene; hydrazino-methylene; $-CH_2$-Het (where Het = a substituted or unsubstituted heterocycle); substituted amino (but when the resulting compound is a secondary alkylamide B must not be phenylalanine); an amino-acid group or its ester or amide; a carboxylic secondary amide or primary amide, when B must be cyclohexyl-alanine or adamantylalanine or other bulky lipophilic, non-aromatic amino-acid (not Ala Leu Ile Val Nva Met Nle Phe Tyr Trp Nal (1)); tertiary-carboxamide; carboxy-alkyl group or its ester or amide or amino acyl derivative.

2. Compounds according to claim 1 wherein A is selected from imino-acids, (e.g. D-proline or an analogue of proline e.g. pipecolinic acid, azetidine carboxylic acid); lipophilic amino acids (e.g. DPhe, DCha, DChg); strongly basic amino acids (e.g. D-Arg or a homologue or analogue of Arg, e.g. amidino- or guanidinophenylalanine); or N-alkyl or C_α-alkyl (including benzyl) derivatives thereof.

3. Compounds according to claim 1 or 2 wherein B is selected from L-Phe, L-Cha, L-αNal, L-Tal, L-(4F)Phe L-(NMe)Phe or other substituted phenylalanines; or N-alkyl or C_α-alkyl (including benzyl) derivatives thereof.

4. Compounds according to claim 1, 2, or 3 wherein R^1 is selected from 3-guanidinopropyl or other guanidinoalkyl group, (or an amidinoalkyl or aminoalkyl group), also para- or meta substituted guanidino or amidino-benzyl or protected forms of the above; optionally basic nitrogens are alkylated (Me, Et or other).

5. Compounds according to any preceding claim wherein subject to the provisos in regard to the nature of Y expressed in claim 1, selection for Y is from:-

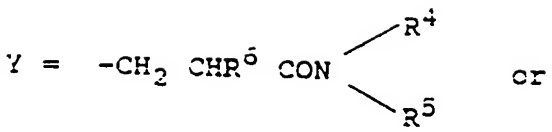
Y = H or alkyl including fluoroalkyl

Y = $-CH_2Q$

where Q = $-OR^2$ or $-SR^2$ or $-SOR^2$ or $-SO_2R^2$ or

$-NHR^2$ or $-N(R^4)(R^5)$ or $-D$

wherein R^2, R^4 and R^5 are as below

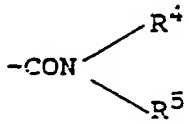


or

wherein R^4, R^5 and R^6 are as below

Y = amino acyl or a group forming a substituted amide or hydrazide

Y = a group forming an α-keto amide - COR^9 or -CO-D or



and in which further:-

R^2 = alkyl or substituted alkyl including aryl or aryl alkyl or $-CH_2R^3$ where R^3 = fluoroalkyl

R^4 and R^5 the same or different but not both hydrogen = H or C_1-C_{20} alkyl (which may be further substituted), acyl or alkyl sulphonyl

-D is a heterocyclic ring (D = nitrogen or carbon in Group IV and N in Groups VI and X) optionally unsaturated and optionally with further hetero atoms and substituents

R^6 = hydrogen, alkyl, hydroxyalkyl, aminoalkyl,

alkylaminocarbonyl

R^9 = $-NH_2$ as such or alkylated, or amino acyl.

6. Compounds according to any claim 1 to 4 wherein subject to the provisos in regard to the nature of Y expressed in claim 1, selection for Y is from:-

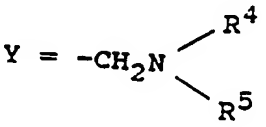
Group I

Y = H; alkyl including branched alkyl (C_1-C_{20}); aryl alkyl; or cycloalkyl (C_1-C_{20}); perfluoroalkyl or partially fluorinated alkyl (C_2-C_{12}); [e.g. Y = Me; $-CH(CH_2CH_2CH_2CH_3)_2$; $-CH(CH_2CH_2CH_2CH_3)CH_2$-cyclohexyl; $-CH_2CF_2CF_2CF_3$; $-CF_2CH_2CH_2CH_3$].

Group II

Y = $-CH_2QR^2$ where Q = O, S, SO, SO_2, NH and where R^2 = Alkyl, branched alkyl or acyl (C_1-C_{12}); or cycloalkyl (C_1-C_{20}); or aryl or aryl alkyl; or $-CH_2-R^3$ where R^3 = perfluoroalkyl or partially fluorinated alkyl, branched or not (C_1-C_{12}).

Group III



R^4, R^5 the same or different = alkyl, branched alkyl, cycloalkyl, acyl, alkylsulphonyl, carboxyalkyl (the carboxyl group may be further derivatized to form an ester or amide with an amino-acid or dipeptide), carbamoyl, sulphamoyl, N-dialkylamino-, arylalkyl, haloalkyl including fluoroalkyl, cyanoalkyl, alkoxyalkyl,

hydroxyalkyl, mercaptoalkyl, aminoalkyl and derivatives thereof e.g. esters, amides and thioesters; or one of R^4 or R^5 = hydrogen

Group IV

$$Y = -CH_2 - \boxed{D}$$

where D = nitrogen or carbon and $-\boxed{D}$ is a saturated or unsaturated heterocyclic ring or a bicyclic ring system, each is 5 - 8 membered, where there may be other hetero-atoms (N, S, O) and carbons or nitrogens may optionally be substituted by alkyl, branched alkyl, cycloalkyl, carboxyalkyl, carboxy (attached to carbon), amino, alkoxy, alkoxymethyl or (carbon) as carbonyl or other groups beneficial for interaction with the enzyme.

Group V

$$Y = -CH_2CH(R^6)CONR^4R^5$$

R^4, R^5 as defined in Group III.

R^6 = hydrogen lower alkyl, branched alkyl, cycloalkyl, hydroxyalkyl, amino-alkyl, alkylaminocarbonyl.

Group VI

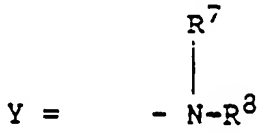
$$Y = -CH_2CH(R^6)CO\boxed{D}$$

R^6 as defined in Group V and $-\boxed{D}$ as defined in Group IV (but with D = N)

Group VII

Y = an amino-acid residue or any amide (secondary or tertiary) or ester of that residue, L or D configuration. Preferred residues are of lipophilic amino-acids e.g. norleucine, cyclohexylalanine, homocyclohexylalanine, cyclohexylglycine, ^{tert}butylglycine.

Group VIII



R^7 = H (when however B is not phenylalanine unless R^8 is carboxylalkyl or derivatized carboxylalkyl); or alkyl, branched alkyl (C_1 - C_{12}), cycloalkyl (C_1-C_{20}) carboxyalkyl or bis(carboxyl)alkyl, which may be derivatized at the carboxyl group to form an amide e.g. with an amino-acid (preferred is arginine) or a substituted amine; N'-dialkylamino; N'-alkylamino-;

R^8 = R^7 the same or different but excluding H.

Group IX

Y = -CO-R^9 but only if B is a bulky non-aromatic lipophilic amino acid or its N^{α} alkyl (C_1 - C_4) derivative (e.g. cyclohexylalanine but excluding Ala, Leu, Ile, Val, Nva, Met, Nle, Phe, Tyr, Trp, Nal(1) and their N-methyl derivatives) where R^9 = NH_2, N'-alkylamino (where the alkyl groups include branched and/or cycloalkyl); an amino-acid residue.

Group X

$$Y = -CO-\overset{\frown}{D}$$

$-\overset{\frown}{D}$ as defined in Group IV (but D = N)

Group XI

$$Y = -CO-NR^4R^5$$

R^4,R^5 as defined in Group III, but not H.

7. Any one of the compounds specifically listed in Tables 1 to 9 herein.

8. A method of treatment (including prophylactic treatment) of an inflammatory or other condition as set out in the indications (1) to (6) herein, particularly an allergic inflammatory condition, wherein an effective amount of a peptide or peptide-analogue kininogenase inhibitor is administered topically or systemically to a patient suffering from or at risk of the condition, the peptide or peptide analogue used being optimally of hexapeptide or smaller size.

9. A method of treatment of the allergic inflammatory phase of asthma, wherein an effective amount of a kininogenase inhibitor e.g. a mast cell tryptase inhibitor is administered topically or systemically to a patient suffering from or at risk of the condition.

10. A method of preparation of a medicament for the topical or systemic treatment (including prophylactic treatment) of

conditions as in claim 8 particularly for allergic inflammatory conditions and especially for asthma as in claim 9, wherein a kininogenase inhibitor is associated in effective amounts with a pharmaceutically acceptable diluent or carrier to constitute said medicament.

11. A method of treatment or of preparation of a medicament as above wherein the inhibitor is a compound as claimed in any of claims 1 to 7.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/01479

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 07 K 5/08 C 07 K 5/02 A 61 K 37/64

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 K A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	US,A,4242329 (CLAESON et al.) 30 December 1980, see the whole document ---	1-11
A	EP,A,0056015 (RYAN, J.W.) 14 July 1982, see page 9, lines 26-28; page 27, lines 20-36 ---	1-11
A	EP,A,0363284 (MERRELL DOW PHARMACEUTICALS) 11 April 1990, see pages 4-5, table II; page 15, lines 4-22 ---	1-11
A	US,A,4835253 (BURTON, J.A.) 30 May 1989, see column 4, line 13 - column 6, line 33 --- -/-	1-11

* Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
16-12-1991	16. 01. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Nicole De Bie

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	Chemical Abstracts, volume 111, 1989, (Columbus, Ohio, US) M.M. Simon et al.: "An inhibitor specific for the mouse T-cell associated serine proteinase 1 (TSP-1) inhibits the cytolytic potential of cytoplasmic granules but not of intact cytolytic T cells", see page 609, abstract 76331q, & J. Cell. Biochem. 1989, 40(1), 1-13	1-11